

**Best Available Copy**

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Thomas D. Madden et al.  
Application No. : 10/788,649  
Filed : February 27, 2004  
For : LIPOSOMAL ANTINEOPLASTIC DRUGS AND USES  
THEREOF

Examiner : Gollamudi S. Kishore  
Art Unit : 1615  
Docket No. : 480208.408D1

Mail Stop RCE  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

DECLARATION OF JAMES H. GOLDIE, M.D.

PURSUANT TO 37 C.F.R. §1.132

I, Dr. James H. Goldie, declare as follows:

1. I am currently a Clinical Emeritus Professor at the University of British Columbia. In addition, I am a consultant to the Cancer Endocrinology Department of the BC Cancer Research Centre, and a medical consultant to INEX Pharmaceuticals Corp., the assignee of this patent application. As detailed in my curriculum vitae, a copy of which is provided as Exhibit A, I received my M.D. from the University of Toronto in 1961. I have held a variety of clinical faculty positions since that time, including serving as the Head of Medical Oncology at the University of British Columbia from 1984 until 1995.

2. I have authored or co-authored over 88 peer-reviewed publications in journals such as Cancer Research, European Journal of Cancer, and the Journal of

Clinical Oncology. I have also co-authored or co-edited a number of books and chapters in books related to cancer treatment.

3. I am familiar with the content of this application, and I have reviewed the Office Action mailed June 9, 2005, including the references cited therein and the Examiner's comments regarding the routes of administration of camptothecins and vinca alkaloids. I submit this Declaration for the purpose of providing evidence to the Examiner that the skilled artisan would not be motivated to administer camptothecins or vinca alkaloids either subcutaneously or intramuscularly, even in light of the references cited by the Examiner.

4. Camptothecins and vinca alkaloids are not approved by the Food and Drug Administration for subcutaneous or intramuscular administration, primarily due to cytotoxic effects associated with such local administration. Local administration of camptothecins, such as topotecan, or vinca alkaloids, such as vincristine, can cause severe inflammation and/or necrosis at the site of administration. Therefore, an oncologist would not consider administering these drugs either subcutaneously or intramuscularly.

5. I further submit that even if liposome encapsulation of camptothecins or vinca alkaloids was able to ameliorate their local toxicity, as has been suggested for vincristine (Boman *et al.*, *Cancer Chemotherapy and Pharmacology* 37: 351-355 (1996); Exhibit B), an oncologist would not administer these drugs in liposomes locally, *i.e.*, either subcutaneously or intramuscularly. Chemotherapy, with agents such as camptothecins and vinca alkaloids, is used in the treatment of disseminated (metastatic) disease or in patients for whom metastasis cannot be excluded; localized cancer is removed surgically or treated with radiation therapy. Accordingly, chemotherapeutic agents are intended to be delivered systemically to ensure access to all possible tumor sites within the body. Liposomal encapsulation allows preferentially drug delivery to tumor sites as a result of liposome extravasation from the blood compartment via leaky

tumor blood vessels. Liposomal drugs are, therefore, administered directly to the blood by intravenous or intra-arterial administration.

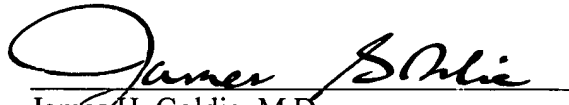
6. Furthermore, for liposomal chemotherapeutic agents, the subcutaneous route would be known to be unsuitable because only a small proportion of the administered liposomes are able to reach the blood compartment and subsequently access tumor sites. For example, Allen *et al.*, (*Biochimica et Biophysica Acta*, 1150: 9-16 (1993); Exhibit C) compared blood and tissue liposome levels following subcutaneous, intravenous, or intraperitoneal administration. These authors showed that liposomes of 110-120 nm diameter or larger were unable to access the blood compartment following subcutaneous administration (<1% of injected dose) (*see* Figure 3, Allen *et al.*). Even small liposomes (80-90 nm), when administered by SC injection achieved only about 30% of the maximum blood levels seen following IV administration, and this modest bioavailability was dependent on the presence of PEG-lipid. Although Allen *et al.* did not specifically evaluate intramuscular injection, this route is similar to subcutaneous administration, since it requires migration of the liposomes via the lymphatic system to lymph nodes and subsequent drainage to the blood compartment. Accordingly, similarly reductions in liposome bioavailability would be expected for the intramuscular route. In view of the fact that liposomes are intended to increase drug delivery to tumor sites, it would not be logical to administer such liposomal formulations of camptothecins or vinca alkaloids by the subcutaneous or intramuscular routes, knowing that this would result in decreased tumor drug levels, since only a minor proportion of the administered liposomes would reach the blood compartment, from which access to tumor sites is achieved.

7. In conclusion, it is my opinion that an oncologist would not administer camptothecins or vinca alkaloids subcutaneously or intramuscularly, whether in free form or encapsulated in liposomes, for the reasons described *supra*. Furthermore, it is my opinion that the references cited by the Examiner and the publications referred to herein do not provide motivation for an oncologist to administer camptothecins or vinca alkaloids subcutaneously or intramuscularly.

I hereby declare that all statements made herein are, to my own knowledge, true and that all statements made on information or belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the captioned patent application or any patent issued therefrom.

Date

OCT. 7 / 2005

  
James H. Goldie, M.D.

# **THE UNIVERSITY OF BRITISH COLUMBIA**

## ***Curriculum Vitae and Teaching Dossier for Faculty Members***

Date: 15 Oct 2002

Initials(signature):

1. SURNAME: Goldie  
FIRST NAME: James  
MIDDLE NAME(S): Hugh
2. DEPARTMENT/SCHOOL: Medicine
3. FACULTY: Medicine
4. PRESENT RANK: Clinical Professor  
SINCE: 1983

### 5. **POST-SECONDARY EDUCATION**

University or Institution	Degree	Dates
University of Toronto	MD	1955 to 1957
University of Toronto	FRCPC	1957 to 1961

### **Special Professional Qualifications**

#### 6. **EMPLOYMENT RECORD**

(a) *Prior to coming to UBC*

University, Company or Organization	Rank or Title	Dates
Royal Marsden Hospital, London, England	Clinical Assistant	1969 - 1970
St. Michael's Hospital, Toronto, Ontario	Staff Physician, Division of Hematology-Oncology	1970 - 1976
Time Department of Medicine, University of Toronto	Assistant Professor, Geographic Full Time	1970 - 1976
B.C. Cancer Agency	Senior Medical Oncologist	1976
Cancer Control Agency of British Columbia	Head, Advanced Therapeutics Department	1977 - 1984
British Columbia Cancer Agency	Head, Division of Medical Oncology	1984 - 1994
British Columbia Cancer Agency	Head, Medical Oncology Laboratory Operations	1991-1995
British Columbia Cancer Agency	Medical Oncologist	1995 - 1998
Inex Pharmaceuticals Corporation	Medical Consultant	1998-present

(b)      *At UBC*

<b>Rank or Title</b>	<b>Dates</b>
Assistant Professor	1976
Clinical Associate Professor	1981
Clinical Prof. & Head, Med Oncology	1984 - 1995
Clinical Professor	1995 - 1998
Clinical Emeritus Professor	1998- present

**THE UNIVERSITY OF BRITISH COLUMBIA  
TEACHING DOSSIER**

**FACULTY OF Medicine**

**DEPARTMENT OR SCHOOL UBC School of Medicine Initial please \_\_\_\_\_**

**A. DESCRIPTION OF INSTRUCTIONAL ACTIVITIES Prepared by Faculty Member.**

**I. INTRODUCTORY INFORMATION**

**Name: Goldie, James Hugh**

**Present Rank: Clinical Professor Medicine**

**Time Period Covered by Dossier (only provide last 5 years) 1991 - 1996**

**Percentage of overall time devoted to:**

- |      |                          |     |
|------|--------------------------|-----|
| i)   | non-clinical instruction | 15% |
| ii)  | clinical instruction     | 5%  |
| iii) | research/publication     | 30% |
| iv)  | administration           | 50% |
| v)   | clinical practice        |     |
| vi)  | other (specify)          |     |

**II. NON-CLINICAL INSTRUCTION**

- a) Classroom Instruction (Lectures, courses, dates, number of students enrolled). Provide titles and number of hours.**

**Rehabilitation Medicine 301, (Basics of Oncology)  
2 to 4 hours Lectures per year**

**Co-Ordinator, Post Graduate Course in Cancer Biology  
3 hours per week during academic year.**

### **III. CLINICAL INSTRUCTION (INCLUDING EXAMINATION TIME)**

#### **TEACHING DOSSIER**

##### **a) RESIDENT/FELLOW**

###### **i) Bedside Teaching**

**1 - 2 hours per month**

###### **ii) Ambulatory Care Teaching**

**1 hour per week**

##### **B. EVALUATION OF INSTRUCTIONAL ACTIVITIES - Supplied by Faculty Member**

#### **TEACHING DOSSIER**

##### **b) Publications and/or professional achievements of graduate students and research fellows.**

**Dr. A.J. Coldman, Head Department of Epidemiology, BCCA.**

**Dr. S. Dedhar, Associate Professor, Cancer Biology, University of Toronto**



8. **TEACHING**

(a) *Courses Taught at UBC:*

Session	Course Number	Scheduled Hours - Lectures
RHSC	301	2 – 4 hrs/wk
MedGen/Pathology	548	3 hrs/wk

9. **SCHOLARLY AND PROFESSIONAL ACTIVITIES**

*Research or equivalent grants (indicate under COMP whether grants were obtained competitively (C) or non-competitively (NC))*

Granting Agency	Subject	Time Period	\$ Awarded
Provincial Government	Anti-cancer Drug Development Program	Oct/92-Mar/95	\$380,000/yr
		Apr/95-Mar/96	\$166,000/yr
National Cancer Institute of Canada	Studies on Methotrexate Resistance	1978- 1990	\$40000/yr
BC Health Care	Math Models of Drug Resistance	1984-88	\$80,000 Total
BC Health Care	Purchase of Coulter Flow Cytometer	1978	\$70000 Total
Pharmaceutical Industry (variable)	Infrastructure support for clinical trials	1984-95	\$50,000/yr (avg)
Provincial Government	Infra Structure Support Grant for Clinical Research Facility	1977- 1995	\$6,000,000 Total
OCTRF	Resistance to Anticancer Agents	1973-76	75,000 Total
MRC	Drug Resistance Circumvention	1970-73	\$8,000/yr

10. **SERVICE TO THE UNIVERSITY**

*Memberships on committees, including offices held and dates*

- Promotions and Tenure Committee, Department of Medicine, 1986 - 1990

11. **SERVICE TO THE COMMUNITY**

(a) *Memberships on scholarly societies, including offices held and dates*

- Chairman, N.C.I.C. Investigational Drug Subcommittee, 1978-81
- Member, N.C.I.C. Investigational Drug Subcommittee, 1981-83
- Cancer Research Coordinating Committee, 1980-82
- Member, Advisory Council on Research, N.C.I.C., 1986-89
- ASCO Education Committee, 1981-83
- Board of Scientific Counselors, Division of Cancer Treatment, U.S. N.C.I., 1981-85.
- Scientific Steering Committee, Frederick Cancer Research Centre, U.S. N.C.I., 1982-85
- Ad Hoc Committee, Drug Screening Program, U.S. N.C.I., 1985-Present
- Scientific Advisory Committee, Eastern Cooperative Oncology Group, 1991-present.

(b) *Editorships (list journal and dates)*

- Cancer Research, 1983-1987
- Current Opinions in Oncology, 1989-present
- Canadian Journal of Oncology, 1991- present

(c) *Reviewer (journal, agency, etc. including dates)*

- OCTRF - Grant Panel, 1974-76
- Scientific Officer, N.C.I.C. Grant Panel C, 1980-82
- Member, N.C.I.C. Grant Panel D, 1985-86
- Cancer Treatment Reports
- Journal Clinical Oncology
- Science
- European Journal of Cancer
- Mathematical Biosciences
- Cancer Chemotherapy and Pharmacology
- Proceedings of the National Academy of Science
- National Science Foundation, Washington, D.C.
- Medical Research Council of Canada
- Alberta Hospitals Cancer Board
- Journal of the National Cancer Institute
- Obstetrics and Gynecology
- Queen Wilhelmina Foundation, The Netherlands
- PSI Foundation, Ontario

12. **AWARDS AND DISTINCTIONS**

*Awards for Scholarship (indicate name of award, awarding organizations, date)*

- Terry Fox Medalist, BCMA, Vancouver, 1982

- Canadian Cancer Society Visiting Professor, University of Alberta, Edmonton and University of Calgary, 1983
- Visiting Professor, Ontario Cancer Treatment and Research Clinic, Hamilton, Ontario, 1984
- Terry Fox Lecturer, Ontario Cancer Institute, Toronto, Ontario, 1984
- W.G. Cosbie Lecturer, Royal College of Physicians and Surgeons of Canada, Toronto, 1990
- First CAMO Lecturer, Toronto, 1990

**THE UNIVERSITY OF BRITISH COLUMBIA**  
***Publications Record***

Date: 11 Feb. 2002

**SURNAME:** Goldie  
**FIRST NAME:** James  
**INITIALS:** H.

1. **REFEREED PUBLICATIONS** (Mark with asterisks [\*] those of primary importance)

*Journals*

**Total first author citations to July 2002 is 2630**

1. \***Goldie, J.H.**, Simmons, A. and Little, J.A.: Crystalline cholesterol: Effect on serum cholesterol levels in patients with hyperlipidemia. *Amer. J. Clin. Nutrition* 22:710-715, 1969. (Peer reviewed)
2. \***Goldie, J.H.** and Osoba, D.: Requirement of a nonproliferating class of cells for generation of immune responses in cell culture. *Proc. Soc. Expl. Biol. & Med.* 133:1265-1269, 1970. (Peer Reviewed)
3. \***Price, L.A.** and **Goldie, J.H.** Multiple drug therapy for disseminated malignant tumours. *Brit. Med. J.* 4:336-339, 1971. (Peer reviewed)
4. \***Goldie, J.H.**, Harrap, K. and Price, L.A.: Methotrexate toxicity: Correlation and duration of administration, plasma levels, dose and excretion pattern. *Europ. J. Cancer* 8:409-414, 1972. (Peer Reviewed)
5. Harrap, K., **Goldie, J.H.**, Hill, B.T. and Price, L.A.: Studies relating to increasing the therapeutic effectiveness of methotrexate. In: *Proc. 7th European Conference on Chemotherapy*, (Seminovsky, ed.), pp. 525-527, Baltimore University Park Publishers, 1972.
6. Pruzanski, W., Warren, R.E., **Goldie, J.H.** and Katz, A.: Malabsorption syndrome with infiltration of the intestinal wall by extracellular monoclonal macroglobulin. *Amer. J. Med.* 54:811-818, 1973. (Peer reviewed)
7. Hill, B.T., Price, L.A. and **Goldie, J.H.**: Studies concerned with over-coming resistance to methotrexate: A comparison of the effects of methotrexate and 2,4-diamino 5 (3',4' dichlorophenyl pyrimidine (BW50197) on the colony-forming ability of L5178Y cells. *Brit. J. Cancer* 28:263-268, 1973. (Peer reviewed)
8. \***Goldie, J.H.**, Furness, M.E. and Price, L.A.: Comparison of the effects of methotrexate and pyrimethamine on L5178Y lymphoblasts in cell culture. *Europ. J. Cancer* 9:709-715, 1973. (Peer reviewed)
9. \***Goldie, J.H.**, Harrison, S.I., Price, L.A. and Hill, B.T.: Impaired responsiveness to folinic acid protection in methotrexate-resistant L5178Y cells. *Europ. J. Cancer* 11:627-632, 1975. (Peer reviewed)
10. Hill, B.T., Price, L.A. and **Goldie, J.H.**: Methotrexate resistance and uptake of DDMP by L5178Y cells. *Europ. J. Cancer* 11:545-553, 1975. (Peer reviewed)

11. Hill, B.T., Price, L.A. and **Goldie, J.H.**: DDMP transport by methotrexate-sensitive and methotrexate-resistant L5178Y cells. Selective protection with folinic acid. Proc. 3rd Congress Europ. Assoc. Cancer Res. 5:12, 1975. (Peer reviewed)
12. Hill, B.T., Price, L.A., Harrison, S.I. and **Goldie, J.H.**: Studies on the transport and distribution of diamino-pyrimidines in L5178Y lymphoblasts in cell culture. Biochem. Pharmacol. 24:535-538, 1975. (Peer reviewed)
13. Price, L.A., **Goldie, J.H.** and Hill, B.T.: Methotrexate as anti-tumour drug. Brit. Med. J. 2:20-21, 1975. (Peer reviewed)
14. Hill, B.T., Price, L.A. and **Goldie, J.H.**: The value of adriamycin in overcoming resistance to methotrexate in tissue culture. Europ. J. Cancer 12:541-549, 1976. (Peer reviewed)
15. Hill, B.T., Price, L.A. and **Goldie, J.H.**: Attempts to overcome methotrexate resistance: The value of drug resistant lines. In: Chemotherapy, Vol. 7, (Hellman and Connor, eds.), pp. 309-314, 1976.
16. \***Goldie, J.H.** and Price, L.A.: Twenty hour combination chemotherapy in advanced breast cancer. Brit. Med. J. 2:1064, 1977. (Peer reviewed)
17. Hill, B.T., Price, L.A., Harrison, S.I. and **Goldie, J.H.**: The differences between selective folinic acid protection and folinic acid rescue in L5178Y cells in culture. Europ. J. Cancer 13:861-871, 1977. (Peer reviewed)
18. Price, L.A., Hill, B.T. and **Goldie, J.H.**: DDMP and selective folinic acid protection in the treatment of malignant disease. Clin. Oncol. 3:281-286, 1977. (Peer reviewed)
19. Price, L.A. and **Goldie, J.H.**: Outpatient chemotherapy for breast cancer. Brit. Med. J. 2(610):1603-1604, 1977. (Peer reviewed)
20. **Goldie, J.H.** and Harrison, S.I.: Comparison of the cytotoxicity of methotrexate and aminopterin on methotrexate resistant murine lymphoma cells in vitro. Europ. J. Cancer 14:55, 1978. (Peer reviewed)
21. **Goldie, J.H.** and Hill, B.T.: An apparent relationship between responsiveness to folinic acid protection from methotrexate cytotoxicity and uptake of 5-methyltetrahydrofolate in a series of murine cell lines. Cancer Biochem. Biophys. 3:111, 1979. (Peer reviewed)
22. \***Goldie, J.H.** and Coldman, A.J.: A mathematical model for relating the drug sensitivity of tumours to their spontaneous mutation rate. Cancer Treat. Rep. 63:1727-1733, 1979. (Peer reviewed)
23. \***Goldie, J.H.**, Krystal, G., Hartley, D., Gudauskas, G. and Dedhar, S.: A methotrexate insensitive variant of folate reductase present in two lines of methotrexate-resistant L5178Y cells. Europ. J. Cancer 16:1539-1546, 1980. (Peer reviewed)
24. **Goldie, J.H.**, Bruchofsky, N., Coldman, A.J. and Gudauskas, G.A.: Rationale for the use of steroid receptors in the adjuvant hormonal therapy of breast cancer. Can. J. Surg. 24:290, 1981. (Peer reviewed)

25. \***Goldie, J.H.**, Dedhar, S. and Krystal, G.: Properties of a methotrexate insensitive variant of dihydrofolate reductase derived from methotrexate resistant L5178Y cells. *J. Biol. Chem.* 256:11629-11635, 1981. (Peer reviewed)
26. Quirt, I., Kersey, P., Baker, M., **Goldie, J.H.** et al: Adjuvant chemotherapy with DTIC and BCG in patients with poor prognosis primary malignant melanoma and completely resected recurrent melanoma. *In: Immunotherapy of Human Cancer*, (Terry and Rosenberg, eds.), pp. 279-283, Elsevier, North Holland/Amsterdam, 1982.
27. **Goldie, J.H.**: Drug resistance and chemotherapeutic strategy. *In: Tumor Cell Heterogeneity: Origins and Implications*, (Owens and Coffey, eds.), pp. 115-125, Academic Press, 1982. (Peer reviewed)
28. Hill, B.T., Dedhar, S. and **Goldie, J.H.**: Evidence that at "High" extra-cellular methotrexate concentrations the transport barrier is unlikely to be an important mechanism of resistance. *Biochem. Pharmacol.* 31(2):263-266, 1982.
29. Dedhar, S., Freisheim, J.H., Hynes, J.B. and **Goldie, J.H.**: Inhibition of a methotrexate-insensitive dihydrofolate reductase from L5178Y cells by substituted triazines and quinazolines. *Biochem. Pharmacol.* 32:922-924, 1983. (Peer reviewed)
30. **Goldie, J.H.**: Drug resistance and cancer chemotherapy strategy in breast cancer. *Breast Cancer Res. and Treat.* 3:129-136, 1983. (Peer reviewed)
31. **Goldie, J.H.**: Cancer: Progress and prospects II - New thoughts on resistance to chemotherapy. *Hospital Practice Series*, 165-177, 1983.
32. \***Goldie, J.H.** and Coldman, A.J.: A quantitative model for multiple levels of drug resistance in clinical tumors. *Cancer Treat. Rep.* 67:923-931, 1983. (Peer reviewed)
33. Quirt, I.C., De Boer, G., Kersey, P.A., Baker, M.A., **Goldie, J.H.** et al: Randomized controlled trial of adjuvant chemimmunotherapy with DTIC and BCG after complete excision of primary melanoma with a poor prognosis or melanoma metastasis. *Can. Med. Assoc. J.* 128:929-933, 1983. (Peer reviewed)
34. \*Coldman, A.J. and **Goldie, J.H.**: A model for the resistance of tumor cells to cancer chemotherapeutic agents. *Math. Biosciences* 65:291-307, 1983. (Peer reviewed)
35. Dedhar, S. and **Goldie, J.H.**: Overproduction of two antigenically distinct forms of dihydrofolate reductase in a highly methotrexate resistant mouse leukemia cell line. *Cancer Res.* 43:4863-4871, 1983. (Peer reviewed)
36. **Goldie, J.H.**: Rationale for the use of alternating chemotherapy as a strategy to deal with drug resistance. *In: Cancer In Ontario. Ninth Clinical Cancer Research Conference*, Geneva Park, Lake Couchiching, Ontario, September 1983.
37. **Goldie, J.H.**: Breast cancer: Why some women respond to chemotherapy, while others don't. *Your Patient and Cancer* 4:59-68, 1984.
38. \***Goldie, J.H.** and Coldman, A.J.: Genetic origin of drug resistance in neoplasms: Implications for systemic therapy. *Cancer Res.* 44:3643-3653, 1984. (Peer reviewed)

39. Dedhar, S., Hartley, D. and **Goldie, J.H.**: Increased dihydrofolate reductase activity in methotrexate-resistant human promyelocytic-leukaemia (HL-60) cells. Lack of correlation between increased activity and over-production. *Biochem. J.* 225:609-617, 1985. (Peer reviewed)
40. Dedhar, S. and **Goldie, J.H.**: Methotrexate-resistant human promyelocytic leukemia (HL-60) cells express a dihydrofolate reductase with altered properties associated with increased enzyme activity. *Biochem. Biophys. Res. Comm.* 129:536-545, 1985. (Peer reviewed)
41. Shah, A., MacDonald, W., **Goldie, J.H.**, Gudauskas, G. and Brisebois, B.: 5-FU infusion in advanced colorectal cancer: A comparison of three dose schedules. *Cancer Treat. Rep.* 69:739-742, 1985. (Peer reviewed)
42. **Goldie, J.H.** and Coldman, A.J.: Genetic instability in the development of drug resistance. *Seminars in Oncology* 12(3):222-230, 1985. (Peer reviewed)
43. Looney, W.B., **Goldie, J.H.**, Little, J.B., Hopkins, H.A., Read, E.J. and Wittes, R.: Alternation of chemotherapy and radiotherapy in cancer management. 1. Summary of the division of cancer treatment workshop. *Cancer Treat. Rep.* 69:769-776, 1985.
44. \*Coldman, A.J., **Goldie, J.H.** and Ng, V.: The effect of cellular differentiation on the development of permanent drug resistance. *Math. Biosciences* 74:177-198, 1985. (Peer reviewed)
45. Coldman, A.J. and **Goldie, J.H.**: Role of mathematical modeling in protocol formulation in cancer chemotherapy. *Cancer Treat. Rep.* 69:1041-1045, 1985. (Peer reviewed)
46. Dedhar, S., Hartley, D., Fitz-Gibbons, D., Phillips, G. and **Goldie, J.H.**: Heterogeneity in the specific activity and methotrexate sensitivity of dihydrofolate reductase from blast cells of acute myelogenous leukemia patients. *J. Clin. Oncol.* 3(11):1545-1552, 1985. (Peer reviewed)
47. **Goldie, J.H.** and Coldman, A.J.: A model for tumor response to chemotherapy: An integration of the stem cell and somatic mutation hypotheses. *Cancer Invest.* 3(6):553-564, 1985. (Peer reviewed)
48. Ragaz, J., Baird, R., Rebbeck, P., **Goldie, J.H.**, Coldman, A. and Spinelli, J.: Neoadjuvant (preoperative) chemotherapy for breast cancer. *Cancer* 56:719-724, 1985. (Peer reviewed)
49. Murray, N., Shah, A., Wilson, K., **Goldie, J.H.**, Voss, N., Fryer, C., Klimo, P., Coy, P., Hadzic, E., Gudauskas, G. and Fowler, R.: Cyclic alternating chemotherapy for small cell carcinoma of the lung. *Cancer Treat. Rep.* 69:1241-1242, 1985. (Peer reviewed)
50. McGuire, W.L., **Goldie, J.H.**, Salmon, S.E. and Ling, V.: View points: Strategies to identify or prevent drug resistance in cancer. *Breast Cancer Res. and Treat.* 5:257-268, 1985.
51. Ragaz, J., Baird, R., Rebbeck, P., Coldman, A. and **Goldie, J.H.**: Neoadjuvant-preoperative-chemotherapy for breast cancer - Preliminary report of the Vancouver trial. In: *Primary Chemotherapy in Cancer Medicine*, (D.J.T.

Wagener, G.H. Blijham, J.B.E. Smeets and J.A. Wils, eds.), pp. 77-87, Alan R. Liss, Inc., New York, 1985.

52. Steinbok, P., Dolman, C.L. and **Goldie, J.H.**: Variation in response to CCNU of glioblastoma multiforme in brain and cervical lymph node. *J. Neurosurg.* 62:918-921, 1985. (Peer reviewed)
53. **Goldie, J.H.**: The genetic basis for tumor heterogeneity: Implications for treatment. *Reviews on Endocrine Related Cancer Supplement* 19:33-37, 1986.
54. **Goldie, J.H.** and Coldman, A.J.: Chemotherapy and autonomy in breast cancer. *Reviews on Endocrine-Related Cancer* 23:17-22, 1986.
55. **Goldie, J.H.** and Coldman, A.J.: Application of theoretical models to chemotherapy protocol design. *Cancer Treat. Rep.* 70:127-131, 1986. (Peer reviewed)
56. Dedhar, S., Freisheim, J.H., Hynes, J.B. and **Goldie, J.H.**: Further studies on substituted quinazolines and triazines as inhibitors of a methotrexate-insensitive murine dihydrofolate reductase. *Biochem. Pharmacol.* 35:1143-1147, 1986. (Peer reviewed)
57. **Goldie, J.H.** and Coldman, A.J.: Analyzing the patterns of treatment failure. (Editorial) *J. Clin. Oncol.* 4:825-826, 1986.
58. **Goldie, J.H.** and Coldman, A.J.: Intrinsic versus acquired drug resistance. (Letter) *Cancer Treat. Rep.* 70:817, 1986.
59. Coldman, A.J. and **Goldie, J.H.**: Role of mathematical modeling in protocol formulation in cancer chemotherapy. (Letter) *Cancer Treat. Rep.* 70:1462, 1986.
60. Coldman, A.J. and **Goldie, J.H.**: A stochastic model for the origin and treatment of tumors containing drug resistant cells. *Bulletin Math. Biol.* 48:279-292, 1986. (Peer reviewed)
61. Murray, N., Shah, A., Brown, E., Kostashuk, E., Laukkunen, E., **Goldie, J.H.**, Band, P., VandenHoek, J., Murphy, K., Sparling, T. and Noble, M.: Alternating chemotherapy and thoracic radiotherapy with concurrent cisplatin-etoposide for limited stage small cell carcinoma of the lung. *Seminars in Oncology* 13:24-30, 1986. (Peer reviewed)
62. **Goldie, J.H.**: Scientific basis for adjuvant and primary (neoadjuvant) chemotherapy. *Seminars in Oncology* 14:1-7, 1987.
63. \*Durand, R.E. and **Goldie, J.H.**: Interaction of etoposide and cisplatin in an in vitro tumor model. *Cancer Treat. Rep.* 71:673-679, 1987. (Peer reviewed)
64. McGuire, W.L., **Goldie, J.H.** Hryniuk, W. and Tormey, D.C.: Drug dosage intensity -a panel discussion. *Breast Cancer Res. and Treat.* 9:87-100, 1987.
65. Coldman, A.J. and **Goldie, J.H.**: Impact of dose intensive chemotherapy on the development of permanent drug resistance. *Seminars in Oncology* 14(4):29-33, 1987. (Peer reviewed)



66. **Goldie, J.H.:** Rationale for "8 in 1" chemotherapy (Letter). *J. Clinical Oncology* 6(2):395, 1988.
67. **Goldie, J.H.:** Mathematical models to predict the behaviour of tumours? *Eur. J. Cancer Clin. Oncol.* 24(4):587-589, 1988. (Peer reviewed)
68. \*Coldman, A.J., Coppin, M.L. and **Goldie, J.H.:** Models for dose intensity. *Mathematical Biosciences* 92:97-113, 1988. (Peer reviewed)
69. McGuire, W., Fojo, A., **Goldie, J.H.** and Ozols, R.: Chemotherapy drug resistance -a panel discussion. *In: Breast Cancer Research and Treatment*, Martinus Nijhoff, Boston, 10:133-144, 1987.
70. **Goldie, J.H.:** Mathematical models of drug resistance and chemotherapy effects. *In: Drug Resistance in Cancer Therapy.* (R. Ozol, ed.), Kluwer Academic Publishers, 13-26, 1989.
71. **Goldie, J.H.:** Mathematical modelling for tumor resistance (Letter). *J. National Cancer Institute* 1988.
72. \***Goldie, J.H.** and Coldman, A.J.: The somatic mutation theory of drug resistance: The Goldie-Coldman Hypothesis Revisited. *P.P.O. Updates*, DeVita V. Jr., Rosenberg S. and Hellman S., eds. Vol. 3,5:1-12, 1989.
73. **Goldie, J.H.:** Chemotherapy sensitivity and resistance in malignancy. *Medicine, North America* 1989;29:5368-5372.
74. Arriagada, R., Bertino, J.R., Bleehen, N.M., Brodin, O., Feld, R., **Goldie, J.H.**, Hansen, H.H., Ihde, D.C., Le Chevalier, T., Souhami, R.L. and Workshop Participants: Consensus report on combined radiotherapy and chemotherapy modalities in lung cancer. *In: Antibiotics Chemother.*, Basel, Karger, 41, 232-241, 1988.
75. **Goldie, J.H.** and Coldman, A.J.: Current status of mutational theories for the origin of drug resistance in neoplasms. *In: Proc VI Intl Conference on Adjuvant Therapy of Cancer*, Salmon S.E. Ed., W.B. Saunders, Philadelphia, 21-25, 1990
76. **Goldie, J.H.** Sequencing of non-cross resistant drugs. (letter) *J Clin Oncol*, in press, 1991.
77. **Goldie, J.H.** Editorial. *Cancer Investigation*, in press, 1991.
78. **Goldie, J.H.**, Ling, V. Evolution of drug resistance. *Can J Oncol*, in press, 1991.
79. Osoba, D., Band, P.R., Connors, J.M., **Goldie, J.H.**, Knowling, M.A., Fetherstonhaugh, E.M.: Treatment of recurrent and metastatic head and neck cancer with cisplatin/etoposide/bleomycin. *Seminars in Oncology* 19(2):25-31, 1992.
80. Osoba, D., Band, P.R., **Goldie, J.H.**, Connors, J.M., Gelmon, K., Knowling, M.A., Fetherstonhaugh, E.M.: Efficacy of weekly cisplatin-based chemotherapy in recurrent and metastatic head and neck cancer. *Annals of Oncology* 3(3):S57-S62, 1992. (Peer reviewed)
81. Embree L., Burns R.B., Heggie J.R., Phillips G.L., Reece D.E., Spinelli J.J., Hartley D.O., Hudon N.J., **Goldie J.H.:** Gas-chromatographic analysis of

busulfan for therapeutic drug monitoring. *Cancer Chemother Pharmacol* 32:137-142, 1993. (Peer reviewed)

82. Jia W, McDermott M, **Goldie J.H.** Cynader M, Tan J, Tufaro F. Selective destruction of gliomas in immunocompetent rats by thymidine kinase-defective herpes simplex virus type 1. *J Natl Cancer Inst* 86:1209-15, 1994.
83. **Goldie J.H.** Arguments supporting the concept of non-cross resistant combinations of chemotherapy. *Cancer Investigation*, 12(3), 324-328 1994. (Peer reviewed)
84. **Goldie J.H.** Modelling the process of drug resistance. *Lung Cancer* 10 Suppl. 1 (1994) S91-S96.
85. Southami RL, Brodin O, Duchesne G, **Goldie J.H.** Green M, Kies M, Mattson K, Mulshine J, Saijo N, Saunders M, Stahel R, Tannock I.: Biological basis of combined therapy. *Lung Cancer* 10 Suppl. 1(1994) S3-S5
86. Embree L, Gelmon KA, Lohr A, Mayer LD, Coldman AJ, Cullis PR, Palaitis W, Pilkievicz F, Hudon NJ, Heggie JR, **Goldie JH.** Chromatographic analysis and pharmacokinetics of liposome-encapsulated doxorubicin in non-small-cell lung cancer patients. *J Pharm Sciences* 1993;82:627-34.
87. Coppin, CML, **Goldie JH.** Adjuvant therapy for breast cancer. *New Eng Jour Med* 331:742 (letter).
88. **Goldie J.H.** DeJong G, Gelmon K, Bally M, Mayer L.: Modulation of Doxorubicin resistance P388VADR cells by R044-5912, a tiapamil derivative, Anti-cancer research Vol.25 No.3, 1995
89. Gleave M, Tolcher A, **Goldie JH.** Progression to androgen independence is delayed by antisense to Bcl-2 in the LnCap mouse prostate model. *Clin Can Res* , 1999
90. Martin Gleave, Hideake Miyake, **Jim Goldie**, Colleen Nelson, Anthony Tolcher. Targeting bcl-2 gene to delay androgen-independent progression and enhance chemosensitivity in prostate cancer using antisense bcl-2 oligodeoxynucleotides. *Urology* 54: 36-46 1999
91. R Klasa, MM B Bally, R Eng, **J H Goldie**, R Gascoyne, F M P Wong. Eradication of Human Non-Hodgkin's Lymphoma in SCID Mice by BCL-2 Antisense Oligonucleotides Combined with Low -Dose cyclophosphamide. *Clin Cancer Res* 6, 2492-2500 2000
92. **J H Goldie** Drug Resistance in Cancer: A Perspective. *Cancer and Metas. Rev.* 20: 63-68, 2001
93. M. Webb, D. Saltman, J. Connors, **J H Goldie.** A Literature Review of Single Agent Treatment of Multiply Relapsed Aggressive Non-Hodgkin's Lymphoma. *Leukemia and Lymphoma*, 43(5) pp. 975-982, 2002

## NATIONAL COMMITTEES

OCTRF - Grant Panel, 1974-76.

Chairman, N.C.I.C. Investigational Drug Subcommittee, 1978-81.

Scientific Officer, N.C.I.C. Grant Panel C, 1980-82.

Member, N.C.I.C. Investigational Drug Subcommittee, 1981-83.  
Cancer Research Coordinating Committee, 1980-82.

Member, N.C.I.C. Grant Panel D, 1985-86.

Member, Advisory Council on Research, N.C.I.C., 1986-89.

## INTERNATIONAL COMMITTEES

ASCO Education Committee, 1981-83.

Board of Scientific Counselors, Division of Cancer Treatment, U.S. N.C.I., 1981-85.

Scientific Steering Committee, Frederick Cancer Research Centre, U.S. N.C.I., 1982-85.

Ad Hoc Committee, Drug Screening Program, U.S. N.C.I., 1985-88

Scientific Advisory Committee, Eastern Cooperative Oncology Group, 1991-94

## 2. NON-REFEREED PUBLICATIONS

### (a) *Journals*

1. **Goldie, J.H.**, Heslop, A. and Little, J.A.: Dietary studies in patients with hyperlipidemia. *Proc. Fed. Biol. Sci.* 1965.
2. **Goldie, J.H.** and Osoba, D.: Cellular interaction during the primary immune response in cell culture. *Proc. Can. Soc. Clin. Invest.* 1969.
3. Osoba, D. and **Goldie, J.H.**: Studies on host immunity. Second Clinical Cancer Conference (OCTRF), 1969.
4. Harrap, K., Furness, M., **Goldie, J.H.** and Price, L.A.: Studies on methotrexate resistant lymphoma cells in vitro. *Proc. Europ. Assoc. Cancer Res.* 2: 1970.
5. Harrap, K., Hill, B.T., Price, L.A. and **Goldie, J.H.**: Attempts to extend the therapeutic use of methotrexate. 7th International Conf. of Chemotherapy 2:715, 1971.
6. Pruzanski, W., Warren, R.E., **Goldie, J.H.** and Katz, A.: Malabsorption associated with infiltration of intestinal wall by a homogenous IgM: A syndrome of macroglobulinosis. *Fed. Proc.* 1972.
7. Hill, B.T., **Goldie, J.H.** and Price, L.A.: Effectiveness of adriamycin in overcoming resistance to methotrexate in L5178Y cells. *Proc. Amer. Assoc. Cancer Res.* 16:44, 1975.

8. Hill, B.T., Price, L.A. and **Goldie, J.H.**: Attempts to overcome resistance to methotrexate: The value of drug resistant cell lines. Proc. 9th International Congress of Chemotherapy, 1975.
9. Hill, B.T., Price, L.A. and **Goldie, J.H.**: DDMP transport by methotrexate-sensitive and methotrexate-resistant L5178Y cells. Selective protection with folinic acid. Proc. 3rd Congress Europ. Assoc. Cancer Res. 5:12, 1975.
10. Price, L.A., Hill, B.T. and **Goldie, J.H.**: Clinical use of DDMP as an antitumour drug. Proc. 9th International Congress of Chemotherapy, London, 1975.
11. Price, L.A., Hill, B.T. and **Goldie, J.H.**: A further report on the clinical use of DDMP in cancer chemotherapy. Proc. 3rd Congress Europ. Assoc. Cancer Res. 5:1, 1975.
12. Hill, B.T., Price, L.A. and **Goldie, J.H.**: Selective folinic acid (CF) protection as opposed to CF rescue in L5178Y cells. Proc. Amer. Assoc. Cancer Res. 18:93, 1977.
13. **Goldie, J.H.**, Garvey, M.B., Bilbao, J., Horsey, W. and Hudson, A.R.: The effect of high dose BCNU adjuvant therapy on the survival of patients with grade III and grade IV astrocytomas. Proc. Royal College of Phys. and Surg. Can. 1978.
14. Gudauskas, G.A. and **Goldie, J.H.**: Pharmacokinetics of high dose 5-FU infusions. Proc. Amer. Assoc. Cancer Res. 19:C-270, 1978.
15. Gudauskas, G.A. and **Goldie, J.H.**: Pharmacokinetics of high dose 5-FU infusions. Annals Royal College of Phys. and Surg. Can. 11:35(47), 1978.
16. Gudauskas, G.A., Hartley, D., Dedhar, S. and **Goldie, J.H.**: Computer assisted data acquisition and processing of FMF (flow microfluorometric) data. Proc. 7th Canadian Medical and Biological Engineering Conference, 1978.
17. **Goldie, J.H.**, Hartley, D., Gudauskas, G. and Dedhar, S.: A methotrexate insensitive form of folate reductase present in methotrexate resistant L5178Y cells. Proc. Amer. Assoc. Cancer Res. 20:15(60), 1979.
18. **Goldie, J.H.** and Coldman, A.J.: Influence of treatment delay on the curability of tumours by adjuvant chemotherapy. Proc. Royal Aust. Coll. Phys. and Surg. 399: 1980.
19. Quirt, I., Kersey, P., Baker, M., **Goldie, J.H.** et al: A comparison of adjuvant chemo-immunotherapy with observation alone in patients with poor prognosis primary malignant melanoma and completely resected recurrent melanoma. Proc. Amer. Soc. Clin. Oncol. 21:C-605, 1980.
20. Shah, A.M., MacDonald, W.C., **Goldie, J.H.**, Gudauskas, G.A. and Sullivan, A.B.: Ambulatory chemotherapy using portable Cormed infusion pump ML6-4. Proc. Amer. Soc. Clin. Oncol. 22:C-796, 1981.
21. Ragaz, J., **Goldie, J.H.**, Coldman, A.J., Baird, R. and Rebbeck, P.: Preoperative adjuvant chemotherapy for cancer of the breast. Can. Soc. Clin. Invest. 1981.
22. Ragaz, J., **Goldie, J.H.**, Coldman, A.J., Baird, R. and Rebbeck, P.: Preoperative adjuvant chemotherapy for cancer of the breast. Clin. Invest. Med. 4:23b, 1981.

23. Ragaz, J., **Goldie, J.H.**, Coldman, A., Baird, R. and Rebbeck, P.: Preoperative adjuvant chemotherapy for cancer of the breast. *Proc. Amer. Soc. Clin. Oncol.* 1:C-337, 1982.
24. Murray, N., Klimo, P., **Goldie, J.H.**, Hodgie, E., Fryer, C., Voss, N. and Gudauskas, G.: Alternating combination chemotherapy for small cell carcinoma of the lung. *Proc. Amer. Soc. Clin. Oncol.* 1:C-587, 1982.
25. Ragaz, J., Baird, R., **Goldie, J.H.**, Rebbeck, P. and Spinelli, J.: Neo-adjuvant-preoperative chemotherapy for breast cancer. *Proc. Amer. Soc. Clin. Oncol.* 2:C-434, 1983.
26. Coldman, A.J. and **Goldie, J.H.**: Role of alternating chemotherapy in the treatment of human cancer. *Proc. Amer. Assoc. Cancer Res.* 24:1082, 1983.
27. Dedhar, S. and **Goldie, J.H.**: Characterization of immunologically isolated dihydrofolate reductase messenger RNA. *UCLA Symposia on Molecular and Cellular Biology - Genome Rearrangement.* *J. Cellular Biochem.* 8B: 139(#1198), 1984.
28. **Goldie, J.H.**, Coldman, A.J., Hopkin, P. and Looney, W.: The alter-nation of chemotherapy and radiotherapy in cancer management. *Proc. Amer. Soc. of Therapeutic Radiologists*, October, 1984.
29. Dedhar, S., Hartley, D., Fitz-Gibbons, D. and **Goldie, J.H.**: Hetero-geneity of dihydrofolate reductase specific activity and methotrexate sensitivity in leukemic blast cells of patients with acute myelogeneous leukemia. *UCLA Symposia on Molecular and Cellular Biology - Leukemia 1985.* *J. Cellular Biochem.* 9:121(#0300), 1985.
30. Coldman, A.J. and **Goldie, J.H.**: A stochastic model for the resistance of tumor cells to cancer chemotherapeutic agents. *Int. Conf. in Statistics, Nagoya, Japan*, 1985.

(b) *Other*

1. Combination Chemotherapy of Advanced Malignancy. Institute of Cancer Research, London, England, May 1971.
2. Multiple Drug Therapy for Resistant Tumours. University of Alberta, Edmonton, May 1973.
3. Effect of Pyrimethamine on Methotrexate Resistant Lymphoma Cells. University of Alberta, Edmonton, May 1973.
4. Chemotherapy of Malignant Lymphomas. American College of Physicians Meeting, Banff, Alberta, June 1975.
5. Effect of Methotrexate on the Cell Cycle. Canadian Society of Cell Biology, Vancouver, B.C., February 1977.
6. Effect of Diaminopyrimidines on Methotrexate Sensitive and Resistant Lymphoma Cells(Report i). Ontario Cancer Treatment and Research Foundation Annual Report, 1974.

7. Effect of Diaminopyrimidines on Methotrexate Sensitive and Resistant Lymphoma Cells(Report ii). Ontario Cancer Treatment and Research Foundation Annual Report, 1975.
8. Effect of Diaminopyrimidines on Methotrexate Sensitive and Resistant Lymphoma Cells (Report iii). Ontario Cancer Treatment and Research Foundation Annual Report, 1976.
9. Folic Acid Antagonists. American College of Physicians Meeting, Pasadena, California, October 1977.
10. Effect of Diaminopyrimidines on Methotrexate Sensitive and Resistant Lymphoma Cells (Report iv). Ontario Cancer Treatment and Research Foundation Annual Report, 1977.
11. Early Treatment of Breast Cancer. Royal Australasian College of Physicians and Surgeons, Sydney, Australia, February 1980.
12. Chemotherapeutic Strategy Based on Mutation to Drug Resistance. Gordon Conference in Experimental Chemotherapy, Plymouth, New Hampshire, July, 1980.
13. Minimizing Drug Resistance in Cancer Chemotherapy. Southern Research Institute, Birmingham, Alabama, October 1980.
14. Chemotherapeutic Strategy and Drug Resistance. Directors Conference, U.S. N.C.I., Bethesda, January 1981.
15. A Mathematical Model of Drug Resistance. Ontario Cancer Institute, Toronto, January 1981.
16. A Computer Model of Chemotherapy Strategy. American Cancer Society Science Writers Symposium, Daytona Beach, Florida, March 1981.
17. Drug Resistance and Cancer Chemotherapy. M.D. Anderson Hospital, Houston, May 1981.
18. Cancer Chemotherapy Strategy and Drug Resistance. Board of Scientific Counsellors, D.C.T. N.C.I., Bethesda, June 1981.
19. Significance of Drug Resistance in the Treatment of Lymphomas and Leukemias. International Summit on Leukemias and Lymphomas, Leeds Castle, England, October 1981.
20. Clinical Implications of the Mutations to Drug Resistance. Imperial Cancer Research Fund Laboratories, London, October 1981.
21. Drug Resistance and Chemotherapy Strategy. Tumour Heterogeneity and Spontaneous Mutations to Drug Resistance. 4th Bristol Myers Symposium on Cancer Research, Baltimore, December 1981.
22. A Quantitative Model for Drug Resistance in Cancer Chemotherapy. UCLA Symposium on Rational Basis for Chemotherapy, Keystone, Colorado, April 1982.
23. Implications of Drug Resistance in Adjuvant Chemotherapy. NSABP Meeting, Hollywood, Florida, May 1982.
24. Mathematical Simulations of Drug Resistance in Tumour Cell Populations. Northern California Cancer Program, San Francisco, CA, May 1982.

25. Computer Assisted Cancer Chemotherapy Strategy. Canada West Medical Congress, 2nd Terry Fox Annual Lecture, Vancouver, B.C., June 1982.
26. Drug Resistance and Adjuvant Chemotherapy. International Conference on Advances in the Adjuvant Therapy of Cancer, London, England, June 1982.
27. Implications of Drug Resistance in the Treatment of Acute Leukemia. St. Jude's Childrens Research Hospital, Memphis, Tennessee, September 1982.
28. Chemotherapeutic Strategy in Breast Cancer. 5th Annual Breast Cancer Symposium, San Antonio, Texas, November 1982.
29. Computer-based Chemotherapy Strategy. Division of Haematology/Oncology, College of Medicine, Cincinnati, Ohio, February 1983.
30. Methotrexate Resistance as a Model of Spontaneous Mutations to Phenotypic Resistance in Tumours. Department of Biochemistry, College of Medicine, Cincinnati, Ohio, February 1983.
31. A Quantitative Model for Multiple Levels of Drug Resistance. Implications for Phase I Testing. N.C.I. Symposium on Drug Resistance, Bethesda, Maryland, February 1983.
32. Computer Simulations of Chemotherapy Strategy. W.W. Cross Cancer Institute, Edmonton, Alberta, April 1983.
33. The Role of Variant Dihydrofolate Reductases in Phenotypic Methotrexate Resistance. W.W. Cross Cancer Institute, Edmonton, Alberta, April 1983.
34. Computer Simulations of Chemotherapy Strategy. Tom Baker Cancer Centre, Calgary, Alberta, April 1983.
35. The Role of Variant Dihydrofolate Reductases in Phenotypic Methotrexate Resistance. Tom Baker Cancer Centre, Calgary, Alberta, April 1983.
36. The Role of Alternating Chemotherapy. OCTRF Conference, Lake Couchiching, Ontario, September 1983.
37. Oncogenes and Implications for Cancer Therapy. David A. Boyes Society Meeting, Vancouver, B.C., November 1983.
38. Combination Radiation and Chemotherapy Treatment Simulations. Radiobiology Workshop, U.S. N.C.I., January 1984.
39. Tumour Heterogeneity: Implications for Therapy. National Bladder Cancer Program Workshop, Sarasota, Florida, January 1984.
40. Tumour Growth Rate and Drug Sensitivity. Ontario Cancer Foundation, Hamilton, Ontario, February 1984.
41. Curability of Cancer by Drugs. Ontario Cancer Foundation, Hamilton, Ontario, February 1984.
42. Basics of Cancer Chemotherapy. Ontario Cancer Institute, Toronto, Ontario, February 1984.
43. Stem Cell Renewal Probability and Drug Resistance. Ontario Cancer Institute, Toronto, Ontario, February 1984.

44. Biology of Anticancer Drug Resistance. W.W. Cross Cancer Institute, Edmonton, Alberta, March 1984.
45. Cell Kinetics and Drug Resistance. W.W. Cross Cancer Institute, Edmonton, Alberta, March 1984.
46. Tumour Growth Rates and Drug Curability. Cell Kinetic Society, Memphis, Tennessee, March 1984.
47. Factors Influencing the Curability of Tumours. London, Ontario, May 1984.
48. Models of Drug Resistance in Cancer Chemotherapy. Wisconsin Cancer Centre, Madison, Wisconsin, June 1984.
49. Drug Resistance In Tumours. ECOG Clinical Meeting, Philadelphia, Pennsylvania, June 1984.
50. Tumour Growth Kinetics. Canadian Society of Laboratory Technologists, Vancouver, B.C., July 1984.
51. Acquired Immune Deficiency Syndrome. College of Family Practice of Canada, Vancouver, B.C., July 1984.
52. Cellular Resistance in Cancer. International Congress of Head and Neck Cancer, Baltimore, Maryland, July 1984.
53. Genetic Basis of Tumour Heterogeneity. 2nd International Conference on Anti-hormones, Berlin, October 1984.
54. Rationale for Preoperative Chemotherapy. 1st International Conference on Chemotherapy Preceding Surgery or Irradiation in Cancer Medicine, Valkenberg, The Netherlands, January 1985.
55. Cell Loss and Drug Resistance. M.D. Anderson Hospital, Houston, Texas, February 1985.
56. Theoretical Considerations in Adjuvant Chemotherapy. 1st International Symposium on Neoadjuvant Chemotherapy, Vancouver, B.C., March 1985.
57. Genetic Basis for Tumour Heterogeneity. 2nd Stanford Symposium on Prostate Cancer, Stanford University, California, March 1985.
58. Tumour Heterogeneity in Implications for Treatment. 2nd Stanford Symposium on Prostate Cancer, Stanford University, California, March 1985.
59. Drug Resistance in Implications for Protocol Design. Children Cancer Study Group, Seattle, Washington, April 1985.
60. Drug Resistance and Cancer Treatment. 2nd International Congress of Advances in Cancer Research, San Remo, Italy, May 1985.
61. Drug Resistance in Tumours. Annual Conference on Medical Oncology, Santa Margherita, Italy, May 1985.
62. Neoadjuvant Chemotherapy. Annual Conference on Medical Oncology, Santa Margherita, Italy, May 1985.



63. Tumour Heterogeneity. Gordon Conference, Cancer, New London, New Hampshire, August 1985.
64. Theoretical Models of Drug Resistance. Symposium on Drug Resistance, Canadian Oncology Society, Vancouver, B.C., September 1985.
65. Genetically Based Resistance and Implications for Cancer Chemotherapy. 1st International Symposium on Chemotherapy, Nagoya, Japan, October 1985.
66. Biology of Cancer. Canadian Society of Laboratory Technicians Course, British Columbia Institute of Technology, Vancouver, B.C., October 1985.
67. The Rationale for Perioperative Chemotherapy Based on the Phenomenon of Drug Resistance. 1st International Congress of Neoadjuvant Chemotherapy, Paris, France, November 1985.
68. Biochemistry of Methotrexate Resistance in Human Leukemia. Department of Biochemistry, Medical College of Toledo, Toledo, Ohio, January 1986.
69. Optimizing Drug Control of Cancer. Symposium on Chemistry and Cancer, Northwest Ohio Cancer Network, Toledo, Ohio, January 1986.
70. Neoadjuvant Chemotherapy of Breast Cancer. Topics in Breast Cancer, Montreal, April 1986.
71. Principles in Neoadjuvant Chemotherapy. ASCO Educational Symposium, May 1986.
72. Basics of Chemotherapy. N.C.I.C. Review Course, Hamilton, Ontario, June 1986.
73. Heterogeneity and Chemotherapy. Gordon Conference, New London, New Hampshire, July 1986.
74. Mathematical Modeling of Drug Resistance. Bristol Myers 9th Symposium on Cancer Research, Washington, D.C., November 1986.
75. Clinical Implications of Drug Resistance. Rutgers University Symposium on Tumour Heterogeneity, New Brunswick, New Jersey, February 1987.
76. Somatic Mutation Theory and Drug Resistance. McGill University, Montreal, Quebec, April 1987.
77. Tumor Growth Rate and Drug Resistance. McGill University, Montreal, Quebec, April 1987.
78. Clinical Trials in Cancer at the B.C. Cancer Agency McGill University, Montreal, Quebec, April 1987.
79. Radiation and Chemotherapy Interactions. IASLC Workshop, Le Havre, France, June 1987.
80. ECOG Retreat on Treatment Strategies in Breast Cancer. Eagle River, Wisconsin, September 1987.
81. Canadian Contributions to Cancer Research. NIH Centennial Meeting, Montreal, Quebec, MRC and McGill, October 1987.
82. Dose Intensity and Drug Resistance. Inter American Congress of Chemotherapy, Clearwater, Florida, January 1988.

83. Models of Tumor Growth Rate and Drug Resistance. University of Maryland, Baltimore, February 1988.
84. New Information on Drug Resistance and Implication for Adjuvant Treatment of Breast Cancer. St. Gallen, Switzerland, March 1988.
85. Circumvention of Drug Resistance in Small Cell Lung Cancer. National Cancer Institute of Japan, Tokyo, April 1988.
86. Drug Resistance. First International Congress on Management of Metastatic Disease. Lyon, France, October, 1988.
87. Theoretical Considerations for More Rational Chemotherapy Protocol Design Hematology/Oncology Conference, The Medical College of Wisconsin, Milwaukee, Wisconsin, November, 1988.
88. Models of Drug Resistance and Implications for Timing of Adjuvant Chemotherapy. Cancer Center Seminar The Medical College of Wisconsin, Milwaukee, Wisconsin, November, 1988.
89. Kinetics of Minimal Disease. International Symposium on Effects of Therapy on Biology and Kinetics of Surviving Tumor Vancouver, B.C., February, 1989
90. Tumour Kinetics and Drug Synergism NSABP Trials Meeting Vancouver, B.C., June, 1989
91. New Concepts in Drug Resistance. VI Conference on Adjuvant Therapy Tucson, Arizona, March, 1990.
92. "Modeling and (Protocol Design)" Royal College of Physicians and Surgeons of Canada Toronto, September, 1990
93. Goldie J.: "Role tumour kinetics and genetic changes in drug resistance: ECOG Annual Meeting, Pittsburgh, USA, June '91
94. Modelling the Program of Drug Resistance IASLC Workshop on Combined Modalities in Lung Cancer, Fontainebleau, France, June '92
95. New directions in cancer treatment. Institut Jules Fabré, Castre, France, Aug.'95
96. Biological aspects of drug resistance. Sunnybrook-Bayview UCTRF Clinic Toronto, Nov.'95
97. University of Kentucky, Lexington, KY, March 1996
  1. Review of Current Concepts of Drug Resistance
  2. Anti-sense and Sensitivity
98. Role of molecular biology in cancer treatment. Royal College of Physicians and Surgeons of Canada, Vancouver, BC, September 1997.
99. Models of drug resistance in Hodgkin's disease. 3rd International Conference on Hodgkin's Disease, Cologne, Germany, March 1998.
100. New Directions in Cancer Research, Canadian Neuro-oncology, Conference, Vancouver, May 2000

### 3. BOOKS

#### (a) *Authored*

**Goldie, J.H.** and Coldman A.J.. Drug Resistance in Cancer: Mechanisms and Models, Cambridge University Press, Cambridge, U.K., 1998.

#### (b) *Edited*

1. Bruchovsky, N. and **Goldie, J.H.** (eds.): Drug and Hormone Resistance in Neoplasia: Basic Concepts, Vol. I, CRC Press, Boca Raton, Florida, U.S.A., 1982.
2. Bruchovsky, N. and **Goldie, J.H.** (eds.): Drug and Hormone Resistance in Neoplasia: Clinical Concepts, Vol. II, CRC Press, Boca Raton, Florida, U.S.A., 1983.
3. Ragaz, J., Band, P. and **Goldie, J.H.** (eds.): Proc. 1st International Symposium of Neoadjuvant (Preoperative) Chemotherapy. Springer-Verlag, New York, 1985 (in press).
4. JH Goldie and AJ Coldman. Drug Resistance in Cancer: Mechanisms and Models. Cambridge University Press, Cambridge, UK. 1998

#### (c) *Chapters*

1. **Goldie, J.H.**: Relevance of drug resistance in cancer treatment strategy. *In*: Cancer Chemotherapy I., (F.M. Muggia, ed.), pp. 1-30, Martinus Nijhoff, 1982.
2. \***Goldie, J.H.**, Coldman, A.J. and Gudauskas, G.A.: A rationale for the use of alternating non-cross-resistant chemotherapy. *Cancer Treat. Rep.* **66**:439-449, 1982. (Peer reviewed)
3. Coldman, A.J. and **Goldie, J.H.**: A mathematical model of drug resistance in neoplasms. *In*: Drug and Hormone Resistance in Neoplasia: Basic Concepts, Vol. I, (N. Bruchovsky and J.H. Goldie, eds.), pp. 55-78, CRC Press, Boca Raton, Florida, 1982.
4. **Goldie, J.H.** and Coldman, A.J.: Clinical implications of the phenomenon of drug resistance. *In*: Drug and Hormone Resistance in Neoplasia: Clinical Concepts, Vol. II, (N. Bruchovsky and J.H. Goldie, eds.), pp. 111-127, CRC Press, Boca Raton, Florida, 1983.
5. Bruchovsky N. and **Goldie, J.H.**: Basis for the use of drug and hormone combinations in the treatment of endocrine-related cancer. *In*: Drug and Hormone Resistance in Neoplasia: Clinical Concepts, Vol. II, (N. Bruchovsky and J.H. Goldie, eds.), pp. 129-162, CRC Press, Boca Raton, Florida, 1983.
6. **Goldie, J.H.**, Coldman, A.J. and Bruchovsky, N.: A quantitative model for drug resistance in cancer chemotherapy. *In*: Rational Basis for Chemotherapy, pp. 23-39. Alan R. Liss Inc., New York, 1983.
7. Ragaz, J., Baird, R., Rebbeck, P., **Goldie, J.H.**, Coldman, A. and Spinelli, J.: Neoadjuvant (preoperative) chemotherapy for breast cancer. *In*: Adjuvant Therapy of Cancer IV, (S.E. Jones and S.E. Salmon, eds.), pp. 425-432, Grune & Stratton, 1984.

8. **Goldie, J.H.:** Influence of tumor growth rate and mutations on drug resistance. In: Head and Neck Cancer, Vol. 1, (P.B. Chretien, M.E. Johns, D.P. Shedd, E.W. Strong and P.H. Ward, eds.), pp. 390-393, C.V. Mosby, St. Louis, 1985.
9. **Goldie, J.H.:** The rationale for the use of preoperative chemotherapy. In: Primary Chemotherapy in Cancer Medicine. Progress in Clinical and Biological Research, Vol. 201, (D. Wagener, et al, eds.), pp. 5-14, Alan R. Liss, Inc., New York, 1985.
10. **Goldie, J.H. and Coldman, A.J.:** Can progression to autonomy be delayed? In: Breast Cancer Treatment & Prognosis, (Basil A. Stoll, ed.), pp. 277-286, Blackwell Scientific Publications, Oxford, 1986.
11. **Goldie, J.H. and Coldman, A.J.:** Theoretical considerations regarding the early use of adjuvant chemotherapy. In: Recent Results in Cancer Research, Vol. 103, (J. Ragaz, P.R. Band and J.H. Goldie, eds.), pp. 30-35, Springer-Verlag, Berlin, Heidelberg, 1986.
12. **Goldie, J.H., Band, P. and Ragaz, J.:** Summary of preoperative (neoadjuvant) chemotherapy. In: Recent Results in Cancer Research, Vol. 103, (J. Ragaz, P.R. Band and J.H. Goldie, eds.), pp. 158-159, Springer-Verlag, Berlin, Heidelberg, 1986.
13. **Coldman, A.J. and Goldie, J.H.:** Factors affecting the development of permanent drug resistance and its impact upon neoadjuvant chemotherapy. In: Recent Results in Cancer Research, Vol. 103, (J. Ragaz, P.R. Band and J.H. Goldie, eds.), pp. 69-78, Springer-Verlag, Berlin, Heidelberg, 1986.
14. **Goldie, J.H.:** Genetically based resistance and its implications for cancer treatment. In: Cancer Chemotherapy: Challenges for the Future, (K. Kimura, K. Yamada, I.H. Krakoff and S.K. Carter, eds.), pp. 28-36, Excerpta Medica, Tokyo, 1986.
15. **Goldie, J.H.:** The rationale for perioperative chemotherapy based on the phenomenon of drug resistance. In: Neo-adjuvant Chemotherapy, Vol. 137, (C. Jacquillat, ed.), pp. 23-28, John Libbey Eurotext Ltd., London, 1986.
16. **Hill, B.T., Whelan, R.D.H., Goldie, J.H. and Dedhar, S.:** Methotrexate resistance in murine and human continuous tumour cell lines: An examination of the different mechanisms involved depending on the order of resistance expressed. In: Progress in Clinical and Biological Research, Vol. 223, Cancer Drug Resistance, (T.C. Hall, ed.), pp. 21-34, Alan R. Liss, Inc., New York, 1986.
17. **Coldman, A.J. and Goldie, J.H.:** Variation in growth parameters and their effect on acquisition of drug resistance. In: Progress in Clinical and Biological Research, Vol. 223, Cancer Drug Resistance, (T.C. Hall, ed.), pp. 103-114, Alan R. Liss, Inc., New York, 1986.
18. **Ragaz, J., Coldman, A., Salinas, F., Worth, A., Manji, M. and Goldie, J.H.:** Neoadjuvant (preoperative) chemotherapy. In: Breast Cancer - Diagnosis and Treatment, (I.M. Ariel and J.B. Cleary, eds.), pp. 312- 327, McGraw-Hill, New York, 1986.
19. **Goldie, J.H.:** Resistance and cancer chemotherapy: General principles. In: Fundamentals of Cancer Chemotherapy, (K. Hellmann and S.K. Carter, eds.), pp. 41-47, McGraw-Hill Book Co., New York, 1987.

20. Coldman, A.J. and **Goldie, J.H.**: Modeling resistance to cancer chemotherapeutic agents. In: Cancer Modeling, (J.R. Thompson and B.W. Brown, eds.), pp. 315-364, Marcel Dekker, Inc., New York, 1987.
21. Ragaz, J., Coldman, A., Salinas, F., Worth, A., Manji, M. and **Goldie, J.H.**: Neoadjuvant (preoperative) chemotherapy. In: Breast Cancer: Diagnosis and Treatment, (I.M. Ariel and J.B. Cleary, eds.), pp. 312- 327, McGraw-Hill Book Company, New York, 1987.
22. **Goldie, J.H.**: Experimental chemotherapy. In: Current Therapy in Hematology/Oncology, pp. 368-370, B.C. Becker, 1987.
23. **Goldie, J.H.**, Coldman, A.J., Ng, V., Hopkins, H.A. and Looney, W.B.: A mathematical and computer-based model of alternating chemotherapy and radiation therapy in experimental neoplasms. In: Treatment Modalities in Lung Cancer, Vol. 41, (R. Arriagada, ed.), pp. 11-20, Antibiotics and Chemotherapy, Basel, Karger, 1988.
24. **Goldie, J.H.** and Coldman, A.J.: Mathematical modeling of drug resistance. In: Mechanisms of Drug Resistance in Neoplastic Cells, pp. 13-28, Academic Press, New York, 1988.
25. **Goldie, J.H.**: New information in drug resistance: Implication for adjuvant treatment of breast cancer. In: Recent Results in Cancer Research, Springer Verlag, 1989;115:8-16.
26. Coldman, A.J. and **Goldie, J.H.**: Drug resistance, heterogeneity and neoadjuvant chemotherapy. In: Proc. 2nd International Congress on Neoadjuvant Chemotherapy, 1989 (in press).
27. **Goldie, J.H.** Genetic and Kinetic Factors in the drug resistance of micrometastatic disease, In: Effects of Therapy on the Biology and Kinetics of the Residual Tumor, Part A, Preclinical aspects. Ragaz J., Simpson-Herron L., Lipmann M., and Fisher B., eds., Wiley-Liss, New York, 99-108, 1990.
28. Coppin, C.M.L., Coldman, A.J., **Goldie, J.H.**: Modeling the effect of chemotherapy intensity. In: Effects of Therapy on the Biology and Kinetics of the Residual Tumor, Part B, Clinical aspects. Ragaz J., Simpson-Herron L., Lipmann M., and Fisher B., eds., Wiley-Liss, New York, 103-116, 1990.
29. **Goldie, J.H.** and Coldman, A.J.: Mathematical modeling of drug resistance. In: Encyclopedia of Human Biology. Dulbecco, R., ed, Academic Press, Orlando, Vol 4, 921-928, 1991.
30. **Goldie J.H.** Drug Resistance. In: The Chemotherapy Source Book, MC Perry, ed, 1992:54-66.
31. **Goldie JH.** Drug resistance: Chemotherapy Source Book, Second Edition, Perry M. Editor, Williams and Wilkins 1996 Chapter 5:63-78.
32. **Goldie JH** Drug Resistance: Chemotherapy Source Book, Third Edition, M Perry ed Williams and Wilkins in press 2001

BBAMEM 76030

## Subcutaneous administration of liposomes: a comparison with the intravenous and intraperitoneal routes of injection

T.M. Allen <sup>a</sup>, C.B. Hansen <sup>a</sup> and L.S.S. Guo <sup>b</sup>

<sup>a</sup> Department of Pharmacology, University of Alberta, Edmonton, AB (Canada) and <sup>b</sup> Liposome Technology Inc., Menlo Park, CA (USA)

(Received 12 February 1993)

Key words: Poly(ethylene glycol); Biodistribution; Liposome; Pharmacokinetics; Subcutaneous administration; Liposome size

The development of long-circulating liposomes containing lipid derivatives of poly(ethylene glycol) (PEG), termed Stealth<sup>®</sup> liposomes, has considerably improved the prospects for therapeutic applications of liposomal drug delivery systems. We have examined the pharmacokinetics and biodistribution of long-circulating, as compared to conventional, liposomes after subcutaneous (sc) administration in mice. Results obtained after subcutaneous administration were compared to those obtained after intravenous (iv) and intraperitoneal (ip) administration. Liposomes, following sc administration, appeared intact in the circulation subsequent to moving down the lymph node chains that drain the site of injection. Liposomes containing PEG-distearoylphosphatidylethanolamine (PEG-DSPE) resulted in the highest levels of small (80–90 nm) liposomes in the blood, with up to 30% of in vivo label appearing in the blood at 12 to 24 h post-injection. In the absence PEG-DSPE approx. 4-fold lower levels of liposomes were found in the blood. Small size of the liposomes was critical to their ability to move into the circulation, with liposomes above 110–120 nm not appearing in blood to any significant extent. The presence of PEG-DSPE and cholesterol was important for the in vivo stability of the liposome after sc administration. Although liposome levels were significantly higher in the draining lymph nodes after sc administration, levels associated with other tissues were proportionately reduced relative to the iv and ip routes of administration. Liposomes appeared in blood after ip and sc administration with half-lives of approx. 0.6 and 9 h, respectively, and subsequent to appearing in blood had similar biodistribution, pharmacokinetics and half-lives (20.4 h) to liposomes given by the iv route.

### Introduction

New long-circulating formulations of liposomes, which contain lipid derivatives of poly(ethylene glycol), are beginning to achieve widespread acceptance as drug delivery systems [1–6]. These liposomes have been termed Stealth<sup>®</sup> <sup>1</sup>, or sterically-stabilized liposomes (S-liposomes) for their ability to avoid detection by the mononuclear phagocyte system (MPS) in vivo [1,7]. Rapid uptake of liposomes by the MPS, and saturation of this important host defense system, has been a significant drawback to liposome formulations used in past studies (reviewed in Ref. 8). Besides their long

circulation half-lives, S-liposomes have the additional advantage of dose-independent pharmacokinetics as a result of their lack of saturation of the MPS in mice at doses as high as 400 mg phospholipid/kg [9].

Several studies have examined the pharmacokinetics and/or biodistribution of S-liposomes after administration by the intravenous (iv) and intraperitoneal (ip) routes [9–13]. Although useful in animal models of disease, the ip route is unlikely to be of much use in human therapy except in specialized circumstances, and the iv route requires a health professional for administration. We were interested in examining the subcutaneous (sc) route of administration of liposomes, not only because it is a simpler route for patient self-administration, but also because it might serve as a depot for the sustained release of drug in vivo. In this study we have compared sc administration of S-liposomes with conventional liposome formulations as a function of liposome dose, size, phospholipid phase transition and the presence or absence of cholesterol.

Correspondence to: T.M. Allen, Department of Pharmacology, University of Alberta, Edmonton, AB, Canada T6G 2H7.

<sup>1</sup> Stealth is a registered trademark of Liposome Technology Inc., Menlo Park, CA, USA.

## Materials and Methods

### Materials

Hydrogenated soy phosphatidylcholine (HSPC), partially hydrogenated phosphatidylcholine with an iodine number of 40 (PC40) [14] and poly(ethylene glycol) (average molecular mass of 1900 daltons) covalently linked via a carbamate bond to distearoylphosphatidylethanolamine (PEG-DSPE) were generous gifts of Liposome Technology Inc. (Menlo Park, CA). The synthesis of PEG-DSPE has been described previously [11]. Phosphatidylglycerol (PG) was purchased from Avanti Polar Lipids (Birmingham, AL). Cholesterol (CHOL) and *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (Tes) were purchased from Sigma (St Louis, MO). Na<sup>125</sup>I was purchased from the Edmonton Radiopharmaceutical Center. Tyraminylinulin was synthesized and <sup>125</sup>I-tyraminylinulin (<sup>125</sup>I-TI) was prepared according to the technique of Sommerman et al. [15]. Pyrogen-free saline (0.9% USP) was obtained from Travenol Canada (Mississauga, Ontario).

### Liposome preparation

Liposomes were extruded multilamellar vesicles (MLV) composed of HSPC or PC(40), with or without CHOL at a 2:1 molar ratio (phospholipid to CHOL) and containing 5 mol% of either PEG-DSPE or PG. PG was substituted for PEG-DSPE in control liposome preparations in order to result in liposomes with the same overall net negative charge. Liposomes were prepared by vortexing dried lipid films in 10 mM Tes-buffered saline, 154 mM NaCl, pH 7.4 (buffer), containing <sup>125</sup>I-TI as an aqueous space label. Liposomes were extruded through two stacked Nuclepore filters from 1.0 to 0.05  $\mu$ m in pore diameter [16,17]. The resulting liposomes were sized by dynamic light scattering using a Brookhaven BI-90 particle sizer (Brookhaven Instruments, Holtsville, NY). Liposome size ranged from 79 to 93 nm for vesicles extruded through 0.05  $\mu$ m filters and 328–718 nm for vesicles extruded through 1.0  $\mu$ m filters. Free <sup>125</sup>I-TI was separated from entrapped label by chromatography over an Ultragel AcA34 column (IBF Biotechnics, France). For doses of 10  $\mu$ mol per mouse, in order to separate free from entrapped label, the liposomes were washed three times with buffer by centrifugation at  $360\,000 \times g$  for 3 h for small liposomes and 1 h for large liposomes. Size distributions were checked before and after the centrifugation step. The average size of the liposomes increased by less than 10% which was not considered significant. For liposomes below 120 nm the polydispersity was low and the liposome preparations appeared as sharp peaks by quasielastic light scattering with an average range of  $\pm 25$  nm. Polydispersity increased rapidly for liposomes of average diameter of

150 nm and above. Phospholipid concentrations were determined by the method of Bartlett [18].

### Biodistribution studies

Female CD<sub>1</sub>(ICR)BR (outbred) mice in the weight range of 23–30 g were obtained from Charles River Canada (St. Constant, Que), and maintained in standard housing. Mice (three per group) were given a single bolus injection with 0.2 ml of liposomes containing approx.  $10^6$  <sup>125</sup>I-TI cpm and either 0.5 or 10  $\mu$ mol phospholipid. Some groups of mice received injections of free <sup>125</sup>I-TI. Injections were either subcutaneous (in the neck or in the upper back just below the neck), intravenous (via the tail vein) or intraperitoneal. After specified periods of time, animals were anaesthetized with halothane (M.T.C. Pharmaceutical, Ontario) and killed by cervical dislocation. Samples of blood (0.1 ml) and internal organs (liver, spleen, lung, kidney, heart, thyroid and carcass, which was the remainder of the animal) were collected. tissues were washed and blotted dry to remove any superficial blood and counted for label in a Beckman 8000 gamma counter. Blood correction factors, having previously been determined from <sup>111</sup>In-labelled red blood cells [7], were applied to tissues and carcass. In some experiments, heparinized blood was collected and blood cells were separated from plasma by 2 min centrifugations in a Microcentrifuge (Model 235B). The cells were washed twice with 1 ml 10 mM Tes-buffered saline and the radioactivity associated with cells or plasma plus washes were counted for <sup>125</sup>I-TI. <sup>125</sup>I-TI is metabolically inert and eliminated rapidly by filtration through the kidneys upon release from liposomes; therefore radiolabel in blood represents intact circulating liposomes and radiolabel in tissues represents liposomes which have been taken up into cells [15,19]. The data is presented as % of in vivo cpm, which represents the % of counts remaining in the body at a given time point. This corrects for leakage of the label from the liposomes and represents intact liposomes remaining in the body. The extent of leakage of label from the circulating liposomes was calculated as the ratio of cpm which remain in the whole animal to injected cpm.

In some experiments additional tissues, including bone marrow and lymph nodes were sampled, as previously described [20]. Results from these experiments are expressed as cpm/mg tissues normalized to  $10^6$  injected cpm and give the relative concentrations of liposomes in each tissue sampled.

### Pharmacokinetics

All pharmacokinetic parameters were calculated from the curve fitting program RSTRIP (Micromath, Salt Lake City, USA). Areas under the time versus concentration curves (AUC) were extrapolated from zero to the last time point, normally 384 h (16 days), or

with  
re:

Re

co  
be  
125

fro

0.2

pc

Th

so

ov

en

lip

di

es

fo

so

fu

cp

je

st

Pl

of

la

in

w

de

at

gi

re

va

fu

bo

T

L

af

(t

R

T

in

1

1

2

4

where blood levels for all liposome compositions had reached zero.

## Results

Following sc administration, radiolabelled liposomes could be found in the blood with peak levels occurring between 6 and 24 h post-injection. When the free  $^{125}\text{I}$ -TI label was injected sc, it was rapidly eliminated from the blood with a half-life of 7.5 min, i.e., less than 0.1% of injected cpm remaining in blood at 12 h post-injection and 2% remained in carcass (Table I). Therefore the radiolabel detected in blood was liposome-entrapped. Peak blood levels of liposomes varied over a large range (Fig. 1A), depending on the presence or absence of PEG-DSPE or cholesterol, and liposome size and dose. Small liposomes (80–90 nm in diameter) containing PEG-DSPE resulted in the highest blood levels (Fig. 1A). When PG was substituted for PEG-DSPE significantly lower blood levels of liposomes were obtained (Fig. 1A). Fig. 1B compares, as a function of liposome composition, the % of injected cpm remaining in the whole animal at 16 days post-injection, which may provide a measure of liposome stability. It is apparent that liposomes containing PEG-DSPE and CHOL resulted in the highest levels of  $^{125}\text{I}$ -TI counts remaining in vivo, while liposomes lacking PEG-DSPE and CHOL resulted in the lowest in vivo levels of  $^{125}\text{I}$ -TI at 16 days post-injection. There was a tendency for the liposomes given at the higher dose of 10  $\mu\text{mol}/\text{mouse}$  to result in higher in vivo cpm at 16 days post-injection than that seen for liposomes given at the lower dose of 0.5  $\mu\text{mol}/\text{mouse}$ , but the results for  $^{125}\text{I}$ -TI cpm at 16 days post-injection did not vary in any significant way with liposome size (Fig. 1B).

Significant trends in blood levels in Fig. 1A, as a function of liposome size, dose and composition, have been presented as a function of time in Fig. 2. Compar-

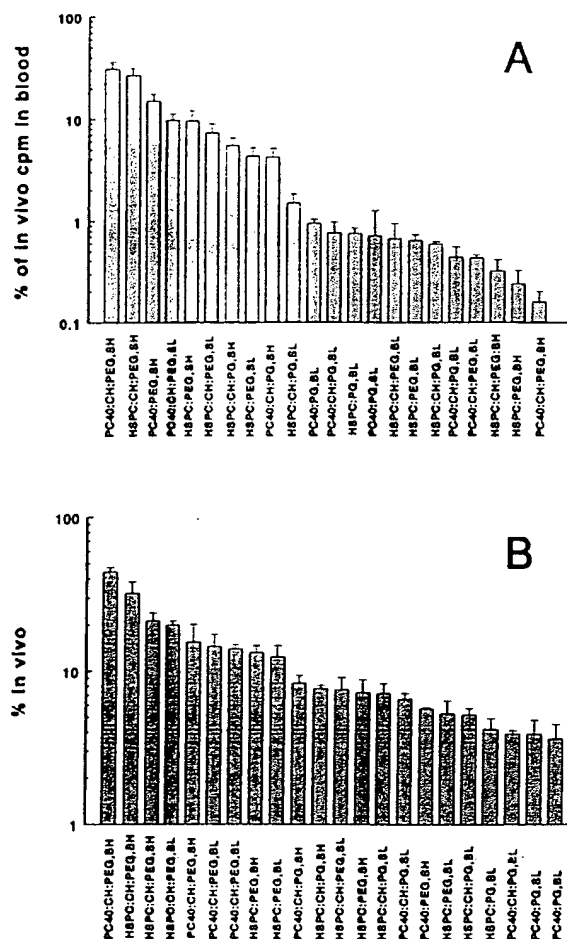


Fig. 1. Mice (three per group) were injected sc in the back of the neck with single doses of various compositions of liposomes, either low (L) dose (0.5  $\mu\text{mol}$  PL/mouse) or high (H) dose (10  $\mu\text{mol}$  PL/mouse). Liposomes were either small (S), with diameters of 79–93 nm, or big (B), with diameters of 328–718 nm. Liposomes were labelled with  $^{125}\text{I}$ -TI. (A) Peak blood levels expressed as % of in vivo cpm  $\pm$  S.D. (B) % of injected cpm remaining in vivo at 16 days post-injection  $\pm$  S.D.

TABLE I

Levels of free  $^{125}\text{I}$ -tyraminylinulin in blood and carcass at various times after subcutaneous administration in the back of the neck in mice (three per group)

Results are expressed as % of injected cpm  $\pm$  S.D.

Time post-injection (h)	% of injected free $^{125}\text{I}$ -tyraminylinulin (cpm $\pm$ S.D.)	
	blood	carcass
0.5	3.7 $\pm$ 0.4	45.4 $\pm$ 3.3
1	2.1 $\pm$ 0.3	39.6 $\pm$ 12.9
2	0.9 $\pm$ 0.3	13.8 $\pm$ 2.4
4	0.4 $\pm$ 0.1	6.6 $\pm$ 1.4
6	0.1 $\pm$ 0.0	3.4 $\pm$ 1.0
12	0.1 $\pm$ 0.0	2.1 $\pm$ 0.3
24	0.1 $\pm$ 0.1	1.9 $\pm$ 0.7
48	0.1 $\pm$ 0.0	1.6 $\pm$ 0.2

ison of blood levels of small liposomes, with or without PEG-DSPE, is given in Fig. 2A. The presence of PEG-DSPE resulted in significantly higher blood levels of liposomes, independent of whether the liposomes were made of saturated (HSPC) or unsaturated (PC40) phospholipids. The presence of cholesterol in the liposomes resulted in higher blood levels of liposomes than when it was absent, both for HSPC-containing liposomes (Fig. 2B) and PC40-containing liposomes, where the results were very similar (not shown). When large liposomes (328–718 nm in diameter) were injected sc, blood levels were significantly lower than when small liposomes were injected (Fig. 2C), and liposomes given at high doses (10  $\mu\text{mol}$  phospholipid/mouse) resulted in higher blood levels than when given at low doses (0.5  $\mu\text{mol}/\text{mouse}$ ) (Fig. 2C). When blood was taken from



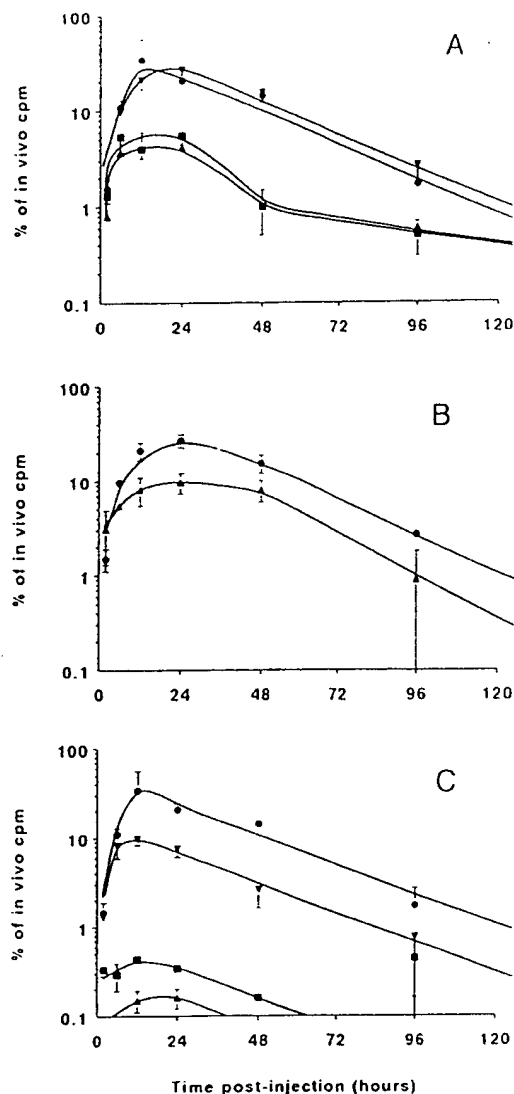


Fig. 2. Blood levels in mice (three per group) for various compositions, sizes and doses of liposomes as a function of time post-injection. Liposomes were labelled with  $^{125}\text{I}$ -TI and results are expressed as % of in vivo cpm  $\pm$  S.D. (A) Liposomes were given at a dose of 10  $\mu\text{mol}/\text{mouse}$ . HSPC/CHOL/PEG-DSPE, average diameter of 89 nm ( $\nabla$ ); PC40/CHOL/PEG-DSPE, 79 nm ( $\bullet$ ); HSPC/CHOL/PG, 74 nm ( $\blacksquare$ ); PC40/CHOL/PG, 90 nm ( $\blacktriangle$ ). (B) Liposomes were given at a dose of 10  $\mu\text{mol}/\text{mouse}$ . HSPC/CHOL/PEG-DSPE, 89 nm ( $\bullet$ ); HSPC/PEG-DSPE, 83 nm ( $\blacktriangle$ ). (C) Liposomes were composed of PC40/CHOL/PEG-DSPE. ( $\bullet$ ) 10  $\mu\text{mol}/\text{mouse}$ , 79 nm; ( $\nabla$ ) 0.5  $\mu\text{mol}/\text{mouse}$ , 82 nm; ( $\blacksquare$ ) 10  $\mu\text{mol}/\text{mouse}$ , 460 nm; ( $\blacktriangle$ ) 0.5  $\mu\text{mol}/\text{mouse}$ , 656 nm.

mice at times corresponding to peak blood levels (12–24 h post-injection) and separated into cellular and plasma fractions, the liposomes were present exclusively (> 99%) in the plasma fraction for liposomes with or without PEG-DSPE (not shown).

Fig. 3 shows, for HSPC/CHOL/PEG-DSPE liposomes, the effect of average liposome diameter on the peak blood levels. It can be seen that there is a

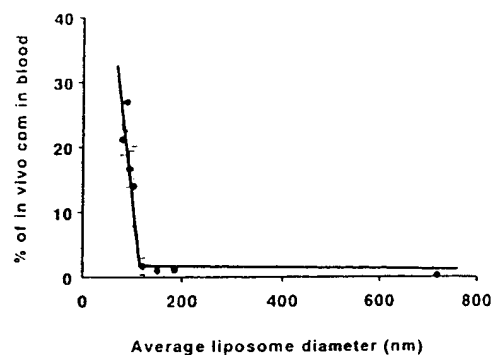


Fig. 3. Maximum blood levels (at 12 or 24 h post-injection) of liposomes (HSPC/CHOL/PEG-DSPE, 2:1:1) in mice (three per group) as a function of average liposome diameter. Results are expressed as % of in vivo cpm  $\pm$  S.D.

dramatic reduction in the levels of liposomes in blood as the size of the liposomes increases. There appears to be a cut-off of approx. 120 nm in diameter above which the liposomes fail to appear in blood to any significant extent. This suggests that the liposomes may be entering the blood, via the lymphatic circulation (see below) through a 'pore' of defined size.

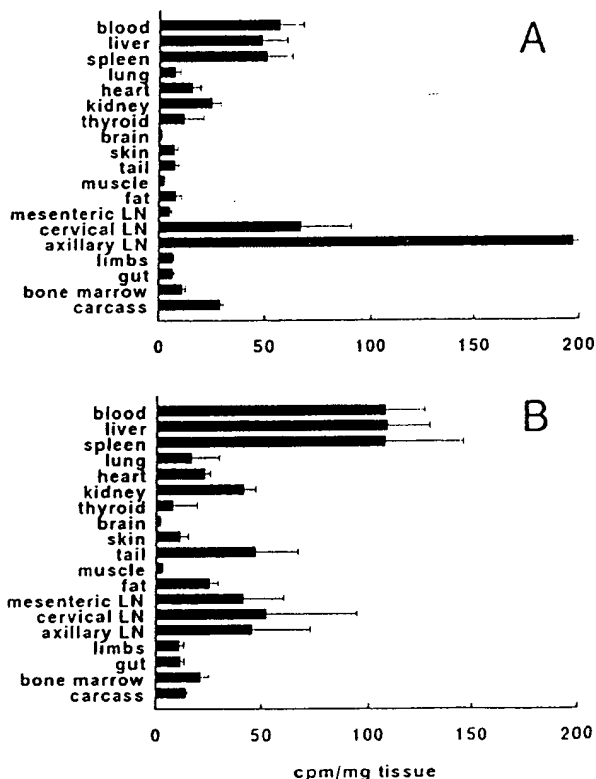


Fig. 4. Tissue distribution in mice (three per group) at 48 h post-injection of PC40/CHOL/PEG-DSPE liposomes (76 nm, 10  $\mu\text{mol}/\text{mouse}$ ) by the sc route (A), or the iv route (B). Results are expressed as cpm/mg tissue  $\pm$  S.D., normalized to  $10^6$  cpm of  $^{125}\text{I}$ -TI injected/mouse. Axillary and brachial lymph nodes are included together under the caption axillary LN.

The tissue distribution of PC40/CHOL/PEG-DSPE liposomes at 48 h following sc administration is shown in Fig. 4A, and Fig. 4B shows their tissue distribution following iv administration for comparison. Following both routes of administration, the distribution of liposomes is widespread throughout the body, and the concentration of liposomes is lower following sc administration than following iv administration in all tissues except in remaining carcass (which contains the site of sc injection), and in the cervical, and axillary and brachial lymph nodes, which drain the region of the injection.

When the distribution of labelled liposomes (PC40/CHOL/PEG-DSPE, 90 nm) was examined in the mesenteric, cervical, combined axillary and brachial lymph nodes, and blood as a function of time after sc administration it can be seen that the mesenteric lymph nodes have low concentrations of liposomes relative to the other two sets of lymph nodes (Fig. 5A). Concentrations of liposomes associated with cervical nodes and with axillary and brachial nodes tended to increase initially and then gradually decreased with time post-

injection (Fig. 5A). A similar trend was observed in blood levels of liposomes with time. This suggests that small S-liposomes are reaching blood by moving down the lymph node chains which drain the site of injection.

For large liposomes in the presence or absence of PEG (PC40/CH/PEG-DSPE, 665 nm or PC40/CH/PG, 444 nm) the levels of liposomes in all sampled lymph nodes and in blood were negligible ( $< 5$  cpm/mg and usually  $< 1$  cpm/mg) suggesting that the large liposomes were not leaving the site of injection to any significant degree (not shown). In the absence of PEG-DSPE, sc injection of small liposomes (PC40/CH/PG, 87 nm) resulted in higher levels of liposomes in lymph nodes, particularly at early times points, and lower blood levels (Fig. 5B). This suggests that, although the liposomes were leaving the site of injection they were being taken up by macrophages or monocytes in lymph nodes to a greater degree than small liposomes containing PEG-DSPE.

Fig. 6 provides a comparison between the iv, ip and sc routes of injection, as a function of time, for blood (Fig. 6A), liver (Fig. 6B), spleen (Fig. 6C), and remaining carcass (Fig. 6D) for liposomes composed of PC40/CHOL/PEG-DSPE (2:1:0.1, molar ratio). Blood levels, after iv injection, followed log-linear pharmacokinetics, as has been previously described [9]. Following both ip and sc injection blood levels initially rose as the liposomes found their way into the blood, and then fell as the liposomes were eliminated from the blood with similar pharmacokinetics as that seen following iv administration of liposomes (Fig. 6A). Blood levels were highest following iv administration, but following ip administration, blood levels approaching iv administration were achieved. Lower blood levels were found after sc administration of liposomes, however, even after sc administration peak blood levels of 30% of in vivo cpm could be achieved (Fig. 1A, Fig. 6A). The principal tissues of liposome uptake were liver and spleen, with levels of uptake being proportional to blood levels following each route of administration, i.e., lower blood levels, and very low liver and spleen levels of liposomes were observed following sc, as compared to iv or ip, administration. Indeed, following sc administration of liposomes, liver levels remained below 10% and spleen levels below 1% of in vivo cpm (Fig. 6B, 6C). Because the carcass contains liposomes residing at the site of ip and sc injection, residual carcass levels initially fell as liposomes were released into blood, and then increased as the liposomes found their way via the bloodstream to other tissues (Fig. 6D).

In Table II a pharmacokinetic comparison is made between the three routes of injection for liposomes of the two compositions which resulted in the highest blood levels following sc administration, HSPC/CHOL/PEG-DSPE (2:1:0.1, molar ratio) and PC40/

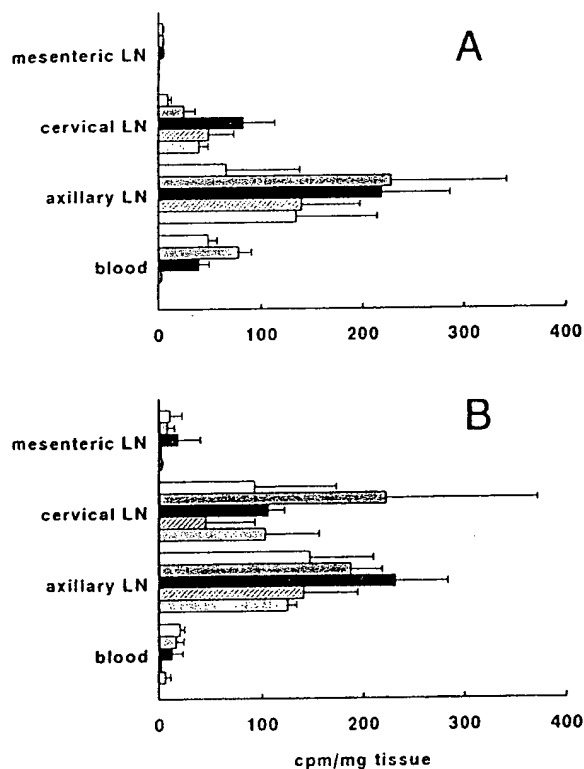


Fig. 5. Lymph node levels of liposomes as a function of time post-injection. Mice received either (A) PC40/CHOL/PEG-DSPE liposomes (90 nm,  $10 \mu\text{mol PL/mouse}$ ) or (B) PC40/CHOL/PG liposomes (87 nm,  $10 \mu\text{mol PL/mouse}$ ) by the sc route of injection. Results are expressed as cpm/mg tissue  $\pm$  S.D., normalized to  $10^6$  cpm of  $^{125}\text{I}$ -TI injected/mouse. Open bars, 12 h post-injection; shaded bars, 24 h; solid bars, 48 h; cross-hatched bars, 96 h; stippled bars, 192 h post-injection.

TABLE II

Pharmacokinetics in blood of liposomes given by three different routes of administration

Liposomes were extruded MLV, 90 nm in diameter. Mice received 10  $\mu$ mol/mouse via the tail vein. The calculation for AUC for free  $^{125}$ I-TI assumes that all of the label was released instantaneously from a theoretical liposome dose of 10  $\mu$ mol/mouse.

Composition	Route of administration	AUC (nmol h/ml)	$T_{1/2\alpha}$	$T_{1/2\beta}$
HSPC/CHOL/PEG-DSPE	iv	355 377		18.0
	ip	362 098	0.8	17.0
	sc	145 453	10.6	19.0
PC40/CHOL/PEG-DSPE	iv	308 758		24.3
	ip	251 123	0.4	24.7
	sc	141 201	7.5	18.2
Free $^{125}$ I-TI	sc	947		0.125

CHOL/PEG-DSPE (2:1:0.1, molar ratio), and for injection of the free  $^{125}$ I-TI label. The area under the blood level vs. time curve (AUC) was very similar following iv and ip administration of liposomes of each composition. Following sc administration, the AUC was reduced approximately in half. The  $T_{1/2\alpha}$ , which provides a measure of the rate of appearance of liposomes in blood after administration by either the ip or sc routes of injection, was on average approx. 0.6 h following ip administration of liposomes and approx. 9

h following sc administration of liposomes of different compositions (Table II). There was a tendency for liposomes containing saturated phospholipids to be released into blood somewhat more slowly (0.8 and 10.6 h for ip and sc injections, respectively) than liposomes containing unsaturated phospholipids (0.4 and 7.5 h for ip and sc injections, respectively) (Table II). Notably, the  $T_{1/2\beta}$ , which provides a measure of the rate of removal of liposomes from the blood, was not significantly different for any of the routes of liposome administration, and averaged  $20.4 \pm 3.4$  h (Table II). This, along with the tissue distribution data of Fig. 4 indicates that the liposomes receive equal treatment once they enter the circulation, regardless of the route of administration.

## Discussion

Small liposomes (< 120 nm in diameter) containing PEG-DSPE, along with their contents, could move to a surprising extent from a sc site of injection into the bloodstream. It appears that movement occurs down the chain of lymph nodes draining the site of injection and that movement from the site of injection into the lymph nodes may occur through a 'pore' which has a maximum diameter of approx. 110–120 nm. The diameter of this 'pore' appears to be similar to that governing migration of particulate matter from the blood through fenestrated endothelium, e.g., to gain access to

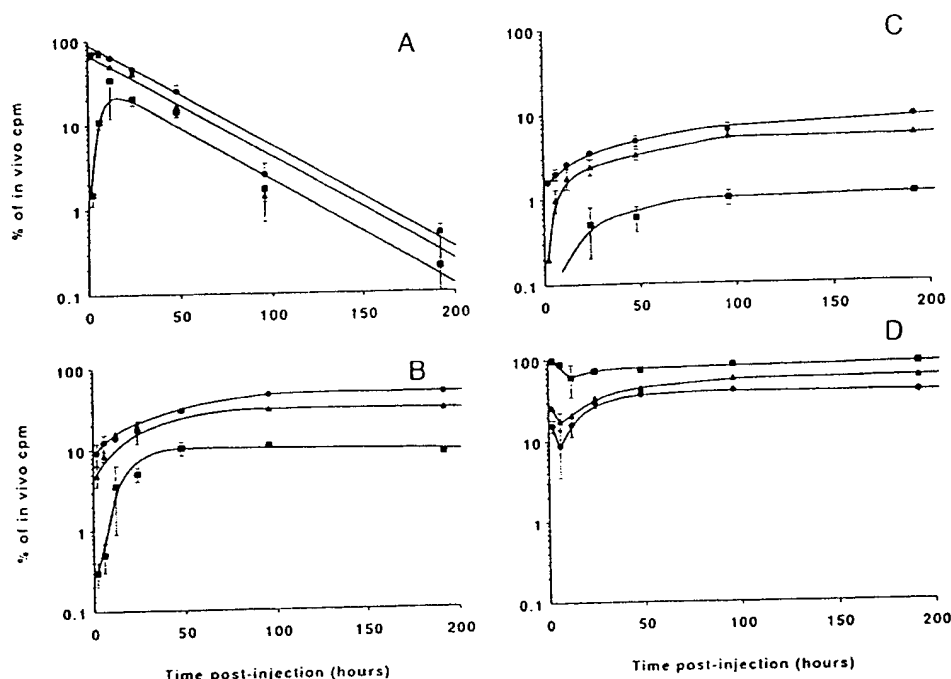


Fig. 6. Tissue distribution of liposomes as a function of iv (●), ip (▲) and sc (■) routes of injection. Liposomes were composed of PC40/CHOL/PEG-DSPE (76 nm, 10  $\mu$ mol/mouse). Results are expressed as % of in vivo cpm  $\pm$  S.D. (A) blood, (B) liver, (C) spleen, (D) carcass.

ferent  
cy for  
to be  
8 and  
1 lipo-  
4 and  
le II).  
e rate  
signif-  
osome  
le II).  
Fig. 4  
tment  
route

aining  
e to a  
to the  
down  
ection  
to the  
has a  
diam-  
overn-  
blood  
ess to

sed of  
n, (D)

liver parenchymal cells. The 'pore' may simply represent the diameter of the interstitial spaces between cells through which liposomes must percolate in order to reach the lymph nodes. Alternative explanations for the size cutoff could include endocytosis of the small liposomes by lymph node macrophages and travel to the blood within these cells, not as free liposomes. However, all liposomes in blood were associated with the plasma fraction, not the cellular fraction. Also, if this were the mechanism, it would be impossible to explain the observation that the pharmacokinetics of small liposomes, once they reach the blood, is identical to free (not cell-associated) liposomes. It is also possible that the small liposomes cross the vascular endothelium by a transcytosis process which might also be size dependent. However, in experiments currently in process in which we are studying the ability of small liposomes to transfer across *in vitro* models of capillary endothelium we find intact, healthy endothelium almost completely impenetrable to liposomes.

Large liposomes, even those containing PEG-DSPE, are not able to enter the circulation to a significant extent: indeed they do not even leave the site of injection. When liposomes lack added PEG-lipid, even those of small size have considerably lower blood levels, likely because they are taken up by macrophages either at the site of injection, or as they move through the lymph nodes, particularly when low doses are given. High doses, which may saturate the ability of lymph nodes to take up the liposomes, appear to increase blood levels. The much higher uptake of PC40/CHOL/PG liposomes into lymph nodes, at the early time points, suggests that these liposomes are being retained in the lymph nodes to a greater extent, contributing to the much lower blood levels seen for small liposomes of this composition as compared to PEG-liposomes. Furthermore, even when the liposomes lacking PEG-DSPE reach blood, they are rapidly removed into liver and spleen, and blood content of these liposomes never reaches high levels. Ingestion of liposomes at the site of injection might then lead to MPS cells percolating down the lymph node chain with their cargo of liposomes. However, even those small PC40/CHOL/PG liposomes which reached blood were associated with the plasma, and not the cellular fraction of blood, which suggests that MPS cells are not a significant part of the mechanism for transport of liposomes to the blood.

The ability of conventional liposomes to be ingested by bone marrow-derived macrophages *in vitro*, and the ability of S-liposomes to avoid this fate has been previously described [21]. This reduced recognition of small PEG-liposomes by MPS cells appears to be the key to their ability to achieve high blood levels after *sc* injection. However, the continued high levels of liposomes in lymph nodes, even at 8 days post-injection (Fig. 5A),

suggests that small PEG-liposomes may be retained in lymph nodes to some extent. *In vivo*, the kinetics of uptake of S-liposomes by MPS cells is much slower than for conventional liposomes and MPS uptake of S-liposomes has been hypothesized to be controlled by the rate at which the PEG is removed from the liposomes resulting in their conversion to conventional liposomes [9].

Less than 1% of injected large liposomes, with or without PEG-lipid, could be found in either liver or spleen, further evidence that they were not able to enter the circulation. We have previously reported that PEG-containing liposomes can reach the blood stream in significant quantities following *ip* administration [11]. Once they reach the circulation after either *sc* or *ip* administration, the liposomes appear to be treated in an identical fashion to liposomes that have been administered by the *iv* route of injection, a fact which can only be explained by the hypothesis that small S-liposomes reach the circulation intact.

We have interpreted the  $^{125}\text{I}$  counts remaining in the body at long time periods post-injection, i.e., 16 days, as providing a possible measure of liposome stability. There are, however, two assumptions associated with this interpretation: the  $^{125}\text{I}$ -TI remains in its original form without degradation, and the label resides in liposomes. Degradation of  $^{125}\text{I}$ -TI, when it leads to release of free  $^{125}\text{I}$ , results in high levels of  $^{125}\text{I}$  associated with thyroid. Although thyroid was one of the tissues sampled in all experiments we have not observed high thyroid levels of label (not shown), suggesting that release of free  $^{125}\text{I}$  from the degradation of  $^{125}\text{I}$ -TI was not a significant problem. Because of the low levels of liposomes and/or label and the difficulties in separating the two at the site of injection, in lymph nodes, etc., it is impossible to determine whether the label was associated with free liposomes or resided within cells at 16 days post-injection, and undoubtedly the data reflect a combination of the two. However, from previous studies on the uptake of conventional and S-liposomes by macrophages [21] it is reasonable to assume that there might be more of a trend towards label being associated with cells in the case of conventional liposomes and towards free liposomes in the case of S-liposomes.

The AUC following *sc* administration of small, PEG-containing liposomes is surprisingly high, and approaches 50% of the AUC found after *iv* or *ip* administration of liposomes of the same composition, size and dose. The AUC for free  $^{125}\text{I}$ -TI, if we assumed for the sake of argument that the label, instead of being injected as free label, was instantaneously released from liposomes upon *sc* injection, was calculated at 47.3 and 947 nmol h/ml for theoretical doses of 0.5 and 10  $\mu\text{mol}$  PL/mouse, respectively. This is only a tiny fraction of the AUC found after injection of liposome-en-

trapped label, and provides a further argument in favour of the label representing intact liposomes. The AUC after sc administration of PEG-containing liposomes of approx. 140000 nmol h/ml, at a dose of 10  $\mu$ mol PL/mouse, can be compared to AUCs of 924 and 89295 nmol h/ml for iv administration of PC/CHOL, 2:1 liposomes (110 nm diameter) at doses of 0.5 and 10  $\mu$ mol/mouse, respectively [9]. In other words, sc administration of small S-liposomes (which have dose-independent pharmacokinetics [9]) resulted in higher AUC than administration of MPS-saturating doses of small conventional liposomes.

An application suggested by the high AUC for the liposomal carrier following sc administration is in the area of depot, sustained drug release, in particular for drugs which are rapidly metabolized when given as free drug. Experiments on the therapeutic efficacy of cytosine arabinoside entrapped in S-liposomes, as compared to conventional liposomes, in the treatment of murine L1210 leukaemia has shown that equivalent increases in mean survival times could be obtained for S-liposomes given by either the sc or iv routes of injection. However, in the case of conventional liposomes the sc route of injection was significantly less efficacious than the iv route [22]. The ability of sc-administered S-liposomes to readily access lymph nodes suggests that there may be applications for antibody-mediated targeting of liposome-entrapped therapeutic drugs to diseased lymph nodes, for example in the case of metastatic cancer.

It has previously been shown that PEG-containing liposomes have significant advantages over conventional liposomes in their long circulation half-lives and dose-independent pharmacokinetics [9]. The ability of sc-administered S-liposomes to move in considerable quantities into the circulation, our ability to manipulate the rate and extent of release into circulation and the liposome stability over a wide range, the high bioavailability of drugs entrapped in these liposomes, along with further reductions in uptake into MPS tissues provide further advantages for PEG-containing liposomes over conventional liposomes.

## Acknowledgements

This work was supported by the Medical Research Council of Canada (MA-9127) and by Liposome Technology Inc., Menlo Park, CA. Jennifer Rutledge provided skilful technical assistance.

## References

- 1 Papahadjopoulos, D., Allen, T.M., Gabizon, A., Mayhew, E., Matthey, K., Huang, S.K., Lee, K.-D., Woodle, M.C., Lasic, D.D., Redemann, C. and Martin, F.J. (1991) *Proc. Natl. Acad. Sci. USA* 88, 11460-11464.
- 2 Allen, T.M., Mehra, R., Hansen, C. and Chin, Y.C. (1992) *Cancer Res.* 52, 2431-2439.
- 3 Gabizon, A., Price, D.C., Huberty, J., Bresalier, R.S. and Papahadjopoulos, D. (1990) *Cancer Res.* 50, 6371-6378.
- 4 Mori, A., Klibanov, A.L., Torchilin, V.P. and Huang, L. (1991) *FEBS Lett.* 284, 263-266.
- 5 Ahmad, I. and Allen, T.M. (1992) *Cancer Res.* 52, 4817-4820.
- 6 Gabizon, A.A. (1992) *Cancer Res.* 52, 891-896.
- 7 Allen, T.M. (1989) in *Liposomes in the Therapy of Infectious Diseases and Cancer* (Lopez-Berestein, G. and Fidler, I., eds.), UCLA Symposium on Molecular and Cellular Biology, Vol. 89, pp. 405-415, A.R. Liss, New York.
- 8 Allen, T.M. (1988) *Adv. Drug. Del. Rev.* 2, 55-67.
- 9 Allen, T.M. and Hansen, C. (1991) *Biochim. Biophys. Acta* 1068, 133-141.
- 10 Gabizon, A. and Papahadjopoulos, D. (1988) *Proc. Natl. Acad. Sci. USA* 85, 6949-6953.
- 11 Allen, T.M., Hansen, C., Martin, F., Redemann, C. and Yau-Young, A. (1991) *Biochim. Biophys. Acta* 1066, 29-36.
- 12 Blume, G. and Cevc, G. (1990) *Biochim. Biophys. Acta* 1029, 91-97.
- 13 Klibanov, A.L., Maruyama, K., Torchilin, V.P. and Huang, L. (1990) *FEBS Lett.* 268, 235-237.
- 14 Lang, J., Vigo-Pelfrey, C. and Martin, F. (1990) *Chem. Phys. Lipids* 53, 91-101.
- 15 Sommerman, E.F., Pritchard, P.H. and Cullis, P.R. (1984) *Biochem. Biophys. Res. Commun.* 122, 319-324.
- 16 Olson, F., Hunt, C.A., Szoka, F.C., Vail, W.J. and Papahadjopoulos, D. (1979) *Biochim. Biophys. Acta* 557, 9-23.
- 17 Mayer, R., Hope, M.J. and Cullis, P.R. (1989) *Biochim. Biophys. Acta* 986, 200-206.
- 18 Bartlett, G.R. (1959) *J. Biol. Chem.* 234, 466-468.
- 19 Allen, T.M. and Chonn, A. (1987) *FEBS Lett.* 223, 42-46.
- 20 Allen, T.M., Hansen, C. and Rutledge, J. (1989) *Biochim. Biophys. Acta* 981, 27-35.
- 21 Allen, T.M., Austin, G.A., Chonn, A., Lin, L. and Lee, K.C. (1991) *Biochim. Biophys. Acta* 1061, 56-64.
- 22 Allen, T.M., Hansen, C.B. and Peliowski, A. (1993) *Drug Target. Deliv.* 1, in press.

## ORIGINAL ARTICLE

Nancy L. Boman · Victor A. Tron · Marcel B. Bally  
Pieter R. Cullis

## Vincristine-induced dermal toxicity is significantly reduced when the drug is given in liposomes

Received: 13 December 1994 / Accepted: 14 May 1995

**Abstract** A problem associated with the intravenous delivery of vincristine concerns drug extravasation at the site of injection or infusion. This can result in extensive local soft-tissue damage. A new formulation of vincristine has recently been developed based on encapsulation of the drug in liposomes. The liposomal drug is somewhat less toxic and substantially more efficacious than free drug. The studies described here assessed, using a murine model of drug extravasation, whether vincristine encapsulation in liposomes influences drug-induced dermal toxicity. It was shown that subcutaneous injection of vincristine in liposomes does not result in the gross skin necrosis and ulceration observed following injection of free drug. Histological analysis of the dermal tissue surrounding the injection site suggests that free drug induces a pronounced inflammatory reaction as judged by the presence of infiltrating leukocytes. In contrast, the liposomal formulation of vincristine engenders a mild prolonged inflammatory condition. These toxicological studies were correlated with an evaluation of drug retention at the site of administration. It was shown using radiolabelled vincristine as a drug marker, that free vincristine is rapidly eliminated from the injection site. In contrast, the level of drug at the site of injection was far greater when the drug was given in liposomal form.

**Key words** Vincristine · Liposomes · Dermal toxicity

### Introduction

Vincristine is a widely used antineoplastic agent that displays effectiveness against a wide variety of neoplasms including Hodgkin's and non-Hodgkin's lymphomas, acute lymphoblastic leukemia, embryonal rhabdomyosarcoma, neuroblastoma, breast carcinoma, and Wilm's tumor [1, 2]. A dose-limiting neurotoxicity is associated with vincristine use, manifested mainly as peripheral neuropathy. Vincristine is also known for its ability to produce soft-tissue necrosis and ulceration if accidentally extravasated during intravenous (i.v.) administration or an inadvertent intramuscular (i.m.) injection [3, 4]. Although several studies have focused on developing and assessing procedures to halt or reverse the necrosis that occurs following an extravasation event [5, 7], there is at present no effective procedure for controlling the outcome after accidental exposure. Further, given the potential hazards associated with vincristine extravasation, the development of treatment protocols based on long-term i.v. infusions have not been developed. This is despite reports strongly suggesting that the therapeutic activity of this cell-cycle-specific drug would be improved significantly if given by long-term infusion [8, 9].

This laboratory has developed a liposomal formulation of vincristine to take advantage of the ability of liposomes to exhibit long residence times in the plasma compartment following i.v. administration [10]. Drug released from circulating liposomes could provide a convenient "micro-infusion" system for this drug. In addition, studies from our laboratory and others have demonstrated that after i.v. administration liposomes containing anticancer agents can accumulate preferentially in regions of disease, such as tumors [11, 12]. Preclinical studies assessing the antitumor activity of this formulation have shown that vincristine is significantly more active when given in liposomal form [10, 13].

The studies described in this report assessed the effect of liposomal encapsulation on vincristine-induced dermal toxicity. It was anticipated, based on previous studies demonstrating reduced dermal toxicity of doxorubicin [14, 15],

N. L. Boman (✉) · P. R. Cullis  
The University of British Columbia, Biochemistry Department,  
2146 Health Sciences Mall, Vancouver, British Columbia,  
Canada V6T 1Z3

V. A. Tron  
Vancouver General Hospital, Department of Pathology, Vancouver,  
British Columbia, Canada

M. B. Bally  
British Columbia Cancer Agency, Division of Medical Oncology,  
600 West 10th Avenue, Vancouver, British Columbia, Canada V5Z 4E6

when given in liposomal form, that liposomal vincristine would not cause ulceration or other damage when given subcutaneously (s.c.) to mice. The studies presented here also determined drug levels within the injection site over time and the results suggest that long-term drug exposure can be achieved safely within defined regions when the drug is given s.c. in liposomal form.

## Materials and methods

Distearoyl phosphatidylcholine (DSPC) was purchased from Avanti Polar Lipids and was > 99% pure. Cholesterol, HEPES, and citric acid were obtained from Sigma (St. Louis, Mo.). Vincristine sulfate was purchased from Lynphomed (Markham, Ont.). [ $^{14}\text{C}$ ]cholesteryl hexadecyl ether was produced by special order from New England Nuclear (Ontario, Canada) and was > 95% pure. It was chosen as a lipid marker due to its stability in vivo [16]. [ $^3\text{H}$ ]Vincristine was obtained from Amersham (Oakville, Ontario, Canada). Female BALB/c mice (retired breeders) were purchased from Charles River Laboratories.

DSPC/Chol (55:45; mol/mol) liposomes were prepared by first dissolving the lipid mixture in 95% ethanol at 60 °C for 30 min (100 mg lipid/ml). Multilamellar vesicles (MLVs) were formed by adding 300 mM citrate, pH 4.0, and vortex mixing vigorously (25 mg lipid/ml final mixture). The resulting MLVs were then incubated at 60 °C for an additional 30 min to ensure equilibration of citrate buffer across the lipid bilayers. Following incubation, large unilamellar vesicles (LUVs) were produced by extruding the MLVs through an extruder containing two Nucleopore polycarbonate filters with 100 nm pore size. The extrusion device was obtained from Lipex Biomembranes (Vancouver, British Columbia, Canada) and was equilibrated at 60 °C. Following extrusion, the liposomes were dialyzed against two changes of 100 volumes of citric acid buffer (pH 4.0) over a 24-h period. Spectra/Por 2 dialysis tubing was used (cutoff 12–14 kDa). The resulting liposomes displayed a mean diameter of 110 nm as demonstrated by quasielastic light scattering.

Vincristine was loaded into the liposomes as follows. The vesicles were passed down a G25 Sephadex column equilibrated with HEPES buffered saline, pH 7.4, to exchange the external buffer. Vincristine sulfate was then added to the liposomes to achieve a drug-to-lipid ratio of 0.1:1. The resulting drug/lipid mixture was then incubated at 60 °C for 10 min. This procedure ensures a > 95% trapping efficiency of the drug [10].

The procedure used for assessing skin toxicity has been previously described [14]. Briefly, an approximately 3-cm<sup>2</sup> area of hair above the hindleg of adult BALB/c mice was removed by vigorous rubbing with Neet topical depilatory lotion (Whitehall Laboratories, New York, N.Y.). This procedure, which causes no adverse skin effects in itself [14], was followed after 24 h by s.c. injection of 10 µg of either free or liposomal vincristine (diluted to 50 µl in normal saline) using a 25-gauge needle (bevel up). Animals were monitored twice daily for any sign of skin irritation or damage. If ulceration was observed the animals were terminated immediately. A more refined assessment of dermal toxicity was based on histologic evaluation of the injection site. Briefly, at selected time points, mice were anesthetized with an intraperitoneal (i.p.) injection of ketamine (160 mg/kg) and xylazine (20 mg/kg) prior to cervical dislocation. Skin around the injection site was removed and placed in 10% formalin. The samples were left in formalin for at least 24 h prior to processing and paraffin embedding. The tissue blocks were cut into 8-µm sections and deparaffinized prior to staining with hematoxylin and eosin.

The level of liposomal lipid and/or vincristine within the injection site was quantified using radiolabelled tracers. Liposomal lipid was measured by incorporating [ $^{14}\text{C}$ ]cholesteryl hexadecyl ether, a non-exchangeable and non-metabolizable lipid marker. Vincristine was measured through use of a [ $^3\text{H}$ ]vincristine label. For these studies, skin around the injection site was removed and subsequently homogenized in saline (0.9%) using a Polytron homogenizer (Brinkmann Instruments, Rexdale, Ont.). The skin homogenates (total volume)



Fig. 1 A, B Female BALB/c mouse given s.c. vincristine either as free drug (A) or in liposomal form (B). Each mouse was given a single s.c. injection (50 µl) of free or liposomal vincristine (10 µg)

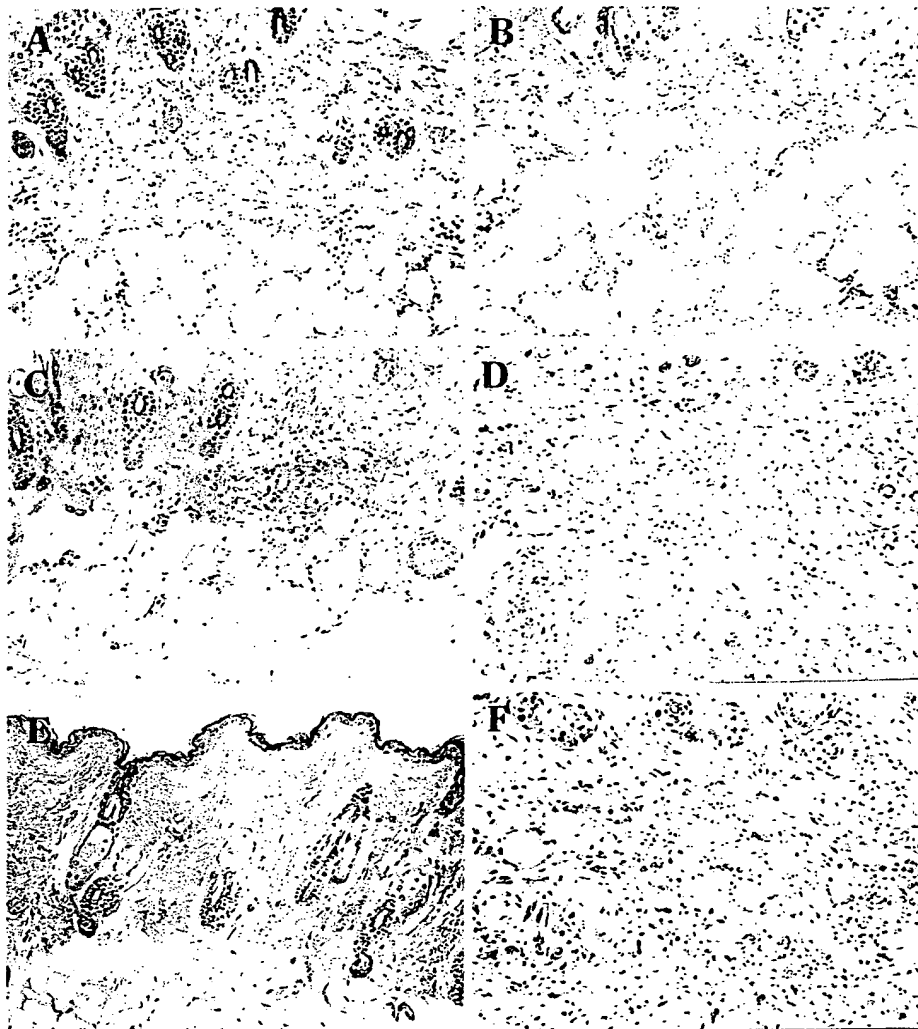
were then digested with 500 µl of "Solvable" (DuPont Canada, Mississauga, Ont.) for 3 h at 50 °C. Subsequently, the samples were cooled to room temperature before decolorizing with 200 µl of 30% hydrogen peroxide. Samples were then analyzed using dual label liquid scintillation counting.

## Results

### Gross ulceration studies

Figure 1A and B are photographs of mice 9 days after being given s.c. injections of free and liposomal vincristine, respectively. Neither treatment group displayed any evidence of erythema or edema at the site of injection over the first 7 days following injection. On day 7, five of the ten mice given free vincristine showed ulcerations with diameters of 2–3 mm. The frequency of ulcerations in mice given free drug was ten/ten with a total of nine mice showing evidence of ulceration by day 10 and all mice ulcerated by day 11. In contrast, mice injected with liposomal vincristine showed no evidence of skin necrosis or ulceration throughout the time course of the experiment.

**Fig. 2 A–F** Histologic skin sections from BALB/c mice given free or liposomal vincristine. Each mouse was given a single s.c. injection (50  $\mu$ l) of free or liposomal vincristine (10  $\mu$ g). All sections are shown at  $\times 100$  magnification and stained with hematoxylin and eosin. **E** Control mice; **A, C** free drug at 1 and 3 days following injection, respectively; **B, D, F** liposomal drug at 1, 3, and 7 days following injection, respectively



Control animals given saline or empty liposomes showed no evidence of an inflammatory response.

#### Histological studies

In an attempt to correlate ulcer formation with more subtle changes in tissue histology, skin sections were examined. Skin was isolated 1, 3, 5, and 7 days after s.c. administration of either free vincristine or liposomal vincristine. The photomicrographs presented here are from representative skin sections. The H&E-stained sections of dermal tissue showed that for animals given free drug there were numerous inflammatory cells in the s.c. injection site within 1 day (Fig. 2A). There were significantly fewer of these cells within sections derived from mice 3 days after drug administration (Fig. 2C). These sections appeared similar to those derived from control animals (Fig. 2E). In contrast, sections obtained from animals given s.c. injections of liposomal vincristine showed a much less intense inflammatory response 1 day after drug administration (Fig. 2B).

However, the presence of inflammatory cells in the dermal area was prolonged, lasting throughout the 7-day study (Fig. 2D,F). In an attempt to quantify the observed changes in inflammatory cells, the number of basophilic cells (blue cells) within selected fields of each section was estimated. Fields were selected on the basis of regions that appeared enriched in inflammatory cells. The results of this analysis are summarized in Table 1. It should be noted that the number of inflammatory cells within the dermal area were relatively constant following s.c. administration of free or liposomal drug. Further, the number of leukocytes within this area was consistent with that observed for control sections. The number of inflammatory cells in the subcutaneous area derived from treated animals confirms the acute and prolonged inflammatory conditions observed following injection of free and liposomal vincristine, respectively. For animals given s.c. injections of liposomal drug there were almost tenfold more inflammatory cells observed on day 7 compared with untreated animals and animals given free drug. As indicated above, no gross lesions were observed in animals given liposomal drug.



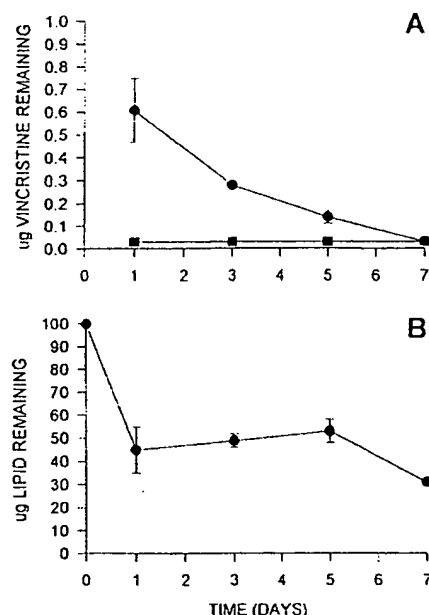
**Table 1** Inflammatory response following subcutaneous injection of free or liposomal vincristine

Injection	Dermal inflammatory cells (cells/mm <sup>2</sup> )	Subcutaneous inflammatory cells (cells/mm <sup>2</sup> )
Controls	$8.2 \times 10^4$	$9.8 \times 10^3$
Free vincristine (10 $\mu$ g)		
Day 1	$1.9 \times 10^5$	$1.3 \times 10^6$
Day 3	$1.8 \times 10^5$	$6.8 \times 10^4$
Day 5	$1.5 \times 10^5$	$9.0 \times 10^4$
Day 7	$1.1 \times 10^5$	$9.0 \times 10^4$
Liposomal vincristine (10 $\mu$ g)		
Day 1	$4.3 \times 10^5$	$6.9 \times 10^5$
Day 3	$2.0 \times 10^5$	$5.4 \times 10^5$
Day 5	$4.6 \times 10^5$	$6.6 \times 10^5$
Day 7	$7.2 \times 10^5$	$9.0 \times 10^5$

These animals were observed for periods in excess of 30 days.

#### Analysis of drug levels within the injection site

In an attempt to establish a pharmacodynamic correlate for drug-induced skin ulceration, the level of drug and/or liposomal lipid was measured within the injection site 1, 3, 5, and 7 days after administration of free or liposomal vincristine. The results, shown in Fig. 3A, indicate that free drug was cleared rapidly from the site of injection. Less than 0.4% of the drug remained at the injection site 1 day after administration. This residual amount of drug, easily detectable by the assay system employed, remained essentially constant over the 7-day time course. In contrast, almost 6% of the drug remained at the injection site 1 day after animals were given liposomal vincristine. This level of drug was more than ten times the level observed after injection of free drug. The level of drug within the injection site gradually declined over the time course of the experiment, with less than 0.3% remaining after 7 days. In addition to monitoring drug levels following s.c. injection of liposomal vincristine, the level of liposomal lipid was determined. It is interesting to note that approximately 45% of the injected lipid dose remained at the injection site 1 day after administration. This level of liposomal lipid was essentially constant throughout the time course. If it is assumed that all the vincristine within the injection site was associated with the liposomal carrier, it can be estimated that the drug-to-lipid ratio decreased approximately 90% within 1 day after administration. This value is comparable to that observed following i.v. administration of the drug, where the drug-to-lipid ratio of liposomes retained in the plasma changes from 0.1 to 0.01 within 24 h [10].



**Fig. 3 A, B** Cutaneous retention of liposomal vincristine. Cutaneous retention of vincristine (A) and lipid (B) following s.c. injection of 10  $\mu$ g of free (■) or liposomal (●) vincristine. The drug-to-lipid ratio of liposomal vincristine was 0.1:1 (w/w). Error bars are the standard deviations of the results from four mice

#### Discussion

This study investigated the effect of liposomal encapsulation on the vesicant properties of vincristine. It has previously been shown that encapsulation of the drug reduces vincristine-induced acute toxicity in mice as evidenced by a decrease in weight loss over time [10, 13]. The soft tissue toxicity of vincristine, however, has received minimal attention until now. In this study we focused on the vesicant properties of the drug since extravasation can seriously affect the patient's quality of life, particularly since vincristine is widely used in pediatric patients for the treatment of acute lymphoblastic leukemia. Extravasation injuries have been shown to be highest in the pediatric and geriatric age groups [17] and has forced the use of more invasive central lines in these patients to administer drug. Extravasation following standard i.v. administration is a relatively common occurrence, occurring in as many as 1–2% of chemotherapy infusions [18]. In patients following the extravasation of the antineoplastic drug doxorubicin, blistering and skin loss become apparent in a few days, followed by progressive tissue necrosis that can continue for as long as 3 months [19]. Full-thickness skin necrosis can eventually ensue exposing underlying tendons and neurovascular structures [18].

The mechanism by which soft-tissue necrosis occurs is widely assumed to be due to a directly cytotoxic effect of the drug. Histologic analysis has been reported on two patients following inadvertent extravasation of cytotoxic

drugs [20, 21]. These studies revealed a nonspecific chronic inflammation with a patent microvasculature. In addition to the gross ulceration observed following s.c. injection of free vincristine using the murine model described here (see Fig. 1A), there were also observed short-term histopathologic changes in the tissue consistent with an inflammatory response (Fig. 2B and Table 1). For these reasons we believe that the murine model used here is representative of the dermal toxicity observed in humans following vincristine extravasation during drug administration. Regardless of the mechanism and severity of vincristine-induced necrosis, various antidote therapies have had questionable efficacy [22, 23].

Since it has been shown previously that liposomal encapsulation of doxorubicin can dramatically reduce the vesicant properties of the drug [15, 24], the use of liposomes to abrogate the vesicant properties of vincristine was assessed. As indicated in Fig. 1B, liposomal encapsulation of vincristine dramatically reduced soft-tissue damage by the drug. There was virtually no evidence of inflammatory response seen grossly when liposomal vincristine was administered s.c. In contrast, free drug produced gross ulceration in 100% of the treated animals within 11 days after injection. Histologic analysis of the injection site suggested that liposomal vincristine induced a mild, but prolonged inflammatory response that was distinct from the intense, short-lived inflammatory response observed after free drug administration. The prolonged inflammatory response observed following s.c. injection of liposomal vincristine may be due to the fact that vincristine levels within the injection site are higher than can be achieved with free drug and are maintained at these levels for periods in excess of 5 days (Fig. 3). The drug leaks slowly from the liposome interior, resulting in the tissue being exposed to a long-term, low dose of free drug. In comparison, when free drug is administered, there is a brief exposure of the tissue to the full drug dose before it is absorbed by the circulatory system. This peak level of exposure to free drug may be the causal factor leading to soft-tissue necrosis.

It is tempting to speculate on the basis of the results presented here that liposomal vincristine could be used safely when treatment consists of local administration. Although it has been suggested that the therapeutic activity of anticancer drugs could be improved through regional delivery [25], clinical studies have shown that unacceptable drug toxicities limit the utility of locally injected free anticancer drugs. It may be possible to avoid these toxicities when the drug is given in liposomal form. The results presented here clearly demonstrate that vincristine-induced dermal toxicity can be reduced when the drug is given in liposomal form. Previous studies from our research group demonstrate that the therapeutic activity of liposomal vincristine is greater than free drug [10, 13]. Therefore, in addition to providing a more potent formulation of the drug, liposomes reduce the potential of vincristine to cause tissue necrosis upon accidental extravasation. This could improve patient quality of life and allow the drug to be administered more safely via standard or alternative routes of administration.

## References

1. Carter SK, Livingston RB (1976) Plant products in cancer chemotherapy. *Cancer Treat Rep* 60: 1141
2. Sieber SM, Mead JAR, Adamson RH (1976) Pharmacology of antitumor agents from higher plants. *Cancer Treat Rep* 60: 1127
3. Bellone JD (1981) Treatment of vincristine extravasation (letter). *JAMA* 245: 343
4. Choy DS (1979) Effective treatment of inadvertent intramuscular administration of vincristine (letter). *JAMA* 241: 695
5. Loth TS, Eversman WW Jr (1986) Treatment methods for extravasations of chemotherapeutic agents: A comparative study. *J Hand Surg* 11A: 388-396
6. Barr RD, Seric J (1981) Soft-tissue necrosis induced by extravasated cancer chemotherapeutic agents: a study of active intervention. *Br J Cancer* 44: 267-269
7. Dorr RT, Alberts DS (1985) Vinca alkaloid skin toxicity: antidote and drug disposition studies in the mouse. *J Natl Cancer Inst* 74: 113-120
8. Jackson DV, White DR, Spurr CL, Hire EA, Pavy MD, Robertson M, Legos HC, McMahon RA (1986) Moderate-dose vincristine infusion in refractory breast cancer. *Am J Clin Oncol* 9: 376-378
9. Jackson DV Jr, Jobson VW, Homesley HD, Welander C, Hire EA, Pavy MD, Votaw ML, Richards F II, Muss HB (1986) Vincristine infusion in refractory gynecologic malignancies. *Gynecol Oncol* 25: 212-216
10. Mayer LD, Bally MB, Loughrey H, Masin D, Cullis PR (1990) Liposomal vincristine preparations which exhibit decreased drug toxicity and increased activity against murine L1210 and P388 tumors. *Cancer Res* 50: 575-579
11. Ogihara I, Kojima S, Jay M (1986) Tumor uptake of 67Ga-carrying liposomes. *Eur J Nucl Med* 11: 405-411
12. Williams BD, O'sullivan MM, Saggu GS, et al. (1987) Synovial accumulation of technetium labelled liposomes in rheumatoid arthritis. *Ann Rheum Dis* 46: 314-318
13. Boman NL, Masin D, Mayer LD, Cullis PR, Bally MB (1994) Liposomal vincristine which exhibits increased drug retention and increased circulation longevity cures mice bearing P388 tumors. *Cancer Res* 54: 2830-2833
14. Dorr RT, Alberts DS, Chen HS (1980) Experimental model of doxorubicin extravasation in the mouse. *J Pharmacol Methods* 4: 237-250
15. Balazsovits JAE, Mayer LD, Bally MB, Cullis PR, McDonall M, Ginsberg RS, Falk RE (1989) Analysis of the effect of liposome encapsulation on the vesicant properties, acute and cardiac toxicities, and antitumor efficacy of doxorubicin. *Cancer Chemother Pharmacol* 23: 81-86
16. Derksen JTP, Morselt HWM, Scherphof GL (1987) Processing of different liposome markers after in vitro uptake of immunoglobulin-coated liposomes by rat liver macrophages. *Biochim Biophys Acta* 931: 33
17. Upton J, Mulliken JB, Murray JE (1979) Major intravenous extravasation injuries. *Am J Surg* 137: 497-506
18. Spiegel RJ (1981) The acute toxicities of chemotherapy. *Cancer Treat Rev* 8: 197-207
19. Reilly JJ, Neifield JP, Rosenberg SA (1977) *Cancer* 40: 2053
20. Rudolph, R, Stein RS, Patillo RA (1976) Skin ulcers due to adriamycin. *Cancer* 38: 1087-1094
21. Chait LA, Dinner MI (1975) Ulceration caused by cytotoxic drugs. *S Afr Med J* 49: 1935-1936
22. Loth TS, Eversman WW Jr (1986) Treatment methods for extravasations of chemotherapeutic agents: a comparative study. *J Hand Surg* 11A: 388-396
23. Dorr RT, Alberts DS (1985) Vinca alkaloid skin toxicity: antidote and drug disposition studies in the mouse. *J Natl Cancer Inst* 74: 113-120
24. Forsen EA, Tokes ZA (1983) Attenuation of dermal toxicity of doxorubicin by liposome encapsulation. *Cancer Treat Rep* 67: 481-484
25. Howell SB, Kirmani S, Goel R (1989) Novel approaches to intraperitoneal drug delivery. *Acta Med Austriaca* 16: 61-64

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☒ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**